THE THALAMUS IN PARKINSON'S DISEASE

A multimodal investigation of thalamic involvement in cognitive impairment

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A thesis submitted in partial fulfilment of the requirements of the

Degree of Doctor of Philosophy in Psychology

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"The thalamus holds the secret of much that goes on within the cerebral cortex,"

Earl Walker, 1938, p.277

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NB: Unless otherwise specified, all brain scans that are displayed throughout this thesis are that of a female PD patient with mild cognitive impairment who, at the time of scanning was 72 years old with 10 years of education and 13 years of disease duration.

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Preface

During the course of this thesis the following aspects of the research were published and presented.

Publications

Abstracts

Borlase, N., Melzer, T., Livingston, L., Graham, C., Pitcher, T., MacAskill, M., Keenan, R., Anderson, T. & Dalrymple-Alford, J. (2011). Diffusion tensor imaging in the thalamus: an advanced method used to investigate cognitive decline in Parkinson's disease (Abstract). *Proceedings of the 29th International Australasian Winter Conference on Brain Research, Queenstown, New Zealand, 29,* Abstract #2.2.

Wang, Y., Borlase, N., Melzer, T., Watts, R., MacAskill, M., Livingston, L., Graham, C., Crucian, G., Anderson, T. & Dalrymple-Alford, J. (2011). Cognitive and neuropsychiatric correlates of medial temporal lobe integrity in Parkinson's disease (Abstract). *Neurodegenerative Diseases*.

Borlase, N., Melzer, T., Watts, R., MacAskill, M., Livingston, L., Graham, C., Pitcher, T., Crucian, G., Keenan, R., Anderson, T. & Dalrymple-Alford, J. (2011). Diffusion tensor imaging and fiber tracking in the thalamus – a new approach to understanding Parkinson's disease (Abstract). *Neurodegenerative Diseases*.

Borlase, N., Melzer, T., Watts, R., Livingston, L., Graham, C., Pitcher, T., Crucian, G., Keenan, R., Anderson, T. & Dalrymple-Alford, J. (2010). Diffusion tensor imaging and fibre tracking applied in the thalamus – a new approach to understanding Parkinson's disease (Abstract). *Proceedings of the 28th International Australasian Winter Conference on Brain Research, Wanaka, New Zealand, 28,* [Abstract #10.1].

Borlase, N., Melzer, T. R., Livingston, L., Graham, C., Keenan, R., Crucian, G., Watts, R., Anderson, T. J. & Dalrymple-Alford, J.C. (2009). Cognitive impairment and dementia in Parkinson's disease: Montreal cognitive assessment and progressive hippocampal volume reduction (Abstract). *Neurodegenerative Diseases, 6 (Suppl. 1),* 169.

Newspaper Articles

Mathewson, N. (2012, September 5). World-first route to identifying dementia in Parkinson's sufferers. *The Christchurch Press,* p9.

Components of this thesis were presented at the following conferences and meetings

Poster Presentations

2012, 24 July, Health Research Society of Canterbury Recycled Poster Evening, University of Canterbury, Christchurch.
2010, 27 August, Van Der Veer Institute Brain Symposium, Don Beaven Medical Research Centre, Christchurch.
2010, 13 March, Alzheimer's and Parkinson's Diseases: Advances, Concepts and New Challenges 9th International Conference, Prague, Czech Republic

Oral Presentations

2012, 21st November, "What If" Lecture Series (Invited Speaker), University of Canterbury, Christchurch.
2012, 14th November, University of Canterbury Showcase, University of Canterbury, Christchurch.
2012, 26th August, Australian Winter Conference on Brain Research, Queenstown.
2012, 22nd August, **'**PhD in 3' Competition, University of Canterbury Final, University of Canterbury, Christchurch
2012, 10 August, 'PhD in 3' Competition, College of Science Final, University of Canterbury, Christchurch
2011, 12 September, 'PhD in 3' Competition, College of Science Final, University of Canterbury, Christchurch.
2011, 28th August, Australasian Winter Conference on Brain Research, Queenstown.
2011, 25th May, New Zealand Federation of Graduate Women Awards Evening, Christchurch.
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2010, 1 September, Australasian Winter Conference on Brain Research, Wanaka, New Zealand.

Accepted Abstracts

Borlase, N., Melzer, T., Watts, R. MacAskill, M.R., Livingston, L., Graham, C., Pitcher, T., Crucian, G., Keenan, R., Anderson, T., Dalrymple-Alford, J., Diffusion Tensor Imaging in the Thalamus - A New Approach to Understanding Parkinson´s Disease accepted for presentation at: Alzheimer's and Parkinson's Diseases: Advances, Concepts and New Challenges 10th International Conference AD/PD 2011; Barcelona, Spain, March 9-13 (Appendix B).

NB: This conference was not attended due to funding constraints

Abstract

Parkinson's disease patients present with the highest risk of dementia development. The thalamus, integral to several functions and behaviours is involved in the pathophysiology of Parkinson's disease. The aim of this thesis was to determine if anatomical abnormalities in the thalamus are associated with the development of dementia in Parkinson's disease.

We examined the thalamus using macro and microstructural techniques and the white matter pathways that connect the thalamus with areas of the surrounding cortex using diffusion tensor imaging (DTI) based tractography. T1-weighted magnetic resonance and DT images were collected in 56 Parkinson's disease patients with no cognitive impairment, 19 patients with mild cognitive impairment, 17 patients with dementia and 25 healthy individuals who acted as control subjects. An established automated segmentation procedure (FIRST FSL) was used to delineate the thalamus and a modified *k*-means clustering algorithm applied to segment the thalamus into clusters assumed to represent thalamic nuclei. Fibre tracts were determined using DTI probabilistic tracking methods available in FIRST. Microstructural integrity was quantified by fractional anisotropy and mean diffusivity (MD) DTI measures.

Results show that microstructural measures of thalamic integrity are more sensitive to cognitive dysfunction in PD than macrostructural measures. For the first time we showed a progressive worsening of cellular integrity (MD) in the groups who had greater levels of cognitive dysfunction. Thalamic degeneration was regionally specific and most advanced in the limbic thalamic nuclei which influenced executive function and attention, areas of cognition that are known to be affected in the earliest stages of PD. The integrity of the fibre tracts corresponding to these thalamic regions was also compromised. Degeneration of fibre tracts was most evident in the dementia group, indicating that they may be more protected against Lewy pathology than the nuclei of the thalamus.

Our findings confirm previous histological, animal and lesion studies and provide a reliable estimate of cortical degeneration in PD that can be applied non-invasively and *in vivo*. A longitudinal study is needed to monitor the progression of cognitive decline in PD but we have provided the basis for further investigation into the predictive validity of thalamic degeneration for cognitive dysfunction. In the future, the microstructural changes of the thalamus could be used as biomarkers for the identification of individuals with a higher risk for dementia development and for the longitudinal monitoring of any interventions into cognitive decline.

List of Main Abbreviations

AD	Alzheimer's disease
AP	Anterior principal nucleus
α-sync	Alpha-synucleun
CL	Central lateral nucleus
CM/Pf	Centromedian/parafascicular nucleus
CSF	Cerebral spinal fluid
CVLT	California Verbal Learning Test
DA	Dopamine
DLB	Dementia with Lewy Bodies
DT	Diffusion tensor
DTI	Diffusion tensor imaging
FA	Fractional anisotropy
fMRI	Functional magnetic resonance imaging
FSL	Functional MRI of the brain (FMRIB) software library
GM	Grey matter
ICV	Intracranial volume
LB	Lewy body
LD	Lateral dorsal nucleus
LN	Lewy neurite
LP	Lateral posterior nucleus
MCI	Mild cognitive impairment
MD	Mean diffusivity
MDn	Mediodorsal nucleus
MDS	Movement Disorder Society
MMSE	Mini Mental State Exam
MNI	Montreal Neurological Institute
MoCA	Montreal Cognitive Assessment
MR	Magnetic resonance
MRI	Magnetic resonance imaging
MS	Multiple sclerosis
NZBRI	New Zealand Brain Research Institute
PD	Parkinson's disease
PD-D	Parkinson's disease with dementia
PD-MCI	Parkinson's disease with mild cognitive impairment
PD-N	Parkinson's disease with no cognitive impairment
Pu	Pulvinar nucleus
RCFT	Rey Complex Figure Test
ROI	Region of Interest
SN	Substantia nigra
UPDRS	Unified Parkinson's disease Rating Scale
VA	Ventral anterior nucleus
VBM	Voxel based morphometry
VL	Ventral lateral nucleus
VP	Ventral posterior nucleus
WM	White matter

Chapter 1. RATIONALE AND HYPOTHESES

1.1 The increasing burden of disease in New Zealand and the cost of dementia

Life expectancy has increased worldwide as we gain better control over infectious diseases and cardiovascular disorders. The additional social and economic cost required to manage the increase in people with age-related decline is substantial. The demand for healthcare has significantly increased and society is now facing the reality of costly long-term care and increased pressure on disability services (Public Health Intelligence *Modelling Stroke: A multi-state life table model.*, 2002). This is a particular concern in New Zealand as the first of the 'baby boomer' population (born 1946-1964) reached retirement age last year and now number 611,400 (as at 30 June 2012), which is projected to increase to 1.2 million in 2036 and 1.5 million in 2061 (Statistics New Zealand, 2008).

As age is the greatest risk factor for many neurological illnesses there is urgent need to address the increasing prevalence of age-related disorders. The increase in neurodegenerative disease is of particular concern as the progressive nature of these disorders dictates unavoidable increases in patient resource allocation as patients require more assistance (McCrone, Allcock, & Burn, 2007). Dementia, the most common neurodegenerative disorder has now reached pandemic levels (Rockwood, Brown, Merry, Sketris, & Fisk, 2002). Without prevention strategies, the number of new dementia cases diagnosed each year in Australia is expected to increase from ~43,000 in 2000 to ~143,000 in 2050 (Jorm, Dear, & Burgess, 2005) and a proportional increase is also expected in New Zealand.

Dementia is the end stage of many different diseases including Alzheimer's disease (AD), Lewy body dementia, frontotemporal dementia and vascular dementia (Bolla, Filley, & Palmer, 2000). Parkinson's disease (PD) is one of the most common neurodegenerative disorders, second only to Alzheimer's disease (Bertram & Tanzi, 2005; de Lau & Breteler, 2006). While recognised as a motor disorder, diagnosis of dementia follows in up to 80% of PD patients (Aarsland, Andersen, Larsen, Lolk, & Kragh -Sorensen, 2003). The prevalence of PD is estimated at 0.08% (76.0 per 100,000) (Caradoc-Davies, Weatherall, Dixon, Caradoc-Davies, & Hantz, 1992) and increases steadily with age to 1% of those over 60 and up to 3% of those over 80 (Tanner & Goldman, 1996). By 2030 the

prevalence of PD is expected to at least double in all developed countries (Dorsey, et al., 2007).

While motor symptoms are the most obvious sign (Hoehn & Yahr, 1967), dementia in PD is the most debilitating symptom and the largest contributor to resource expenditure (Aarsland, Larsen, Karlsen, Lim, & Tandberg, 1999) due to the requirement for nursing home placement as, after age, dementia is the single greatest predictor of nursing home admission for PD patients (Aarsland, Tandberg, & Laake, 2000).

1.2 The need for pre-symptomatic identification of cognitive dysfunction

If dementia onset could be delayed by even 5 years then dementia prevalence could be reduced by 44% (Jorm, et al., 2005). There are several treatment options for PD and for PD dementia (PD-D) which could aid in a worldwide reduction in resource expenditure. The only treatment to be approved by the Food and Drug Administration in the United States is rivastigmine, which acts to increase cholinergic activity (Maidment, Fox, & Boustani, 2010). This has been shown to improve cognition and aspects of daily living (Emre, et al., 2004). Donepezil, another acetylcholinesterase inhibitor, also shows significant promise in treating cognitive dysfunction in Parkinson's disease dementia. After 24 weeks of donepezil treatment, PD-D patients showed improvement on measures of executive function and global cognition (Dubois, et al., 2012). The efficacy of drug treatment increases as a function of early recognition of cognitive decline. Recognising cognitive decline as early as possible is therefore paramount in offering the best treatment (Seltzer, 2006).

The urgency of this requirement was outlined by the National Dementia Strategy for New Zealand which was launched in 2010. The report highlights the importance of detecting the early stages of Alzheimer's dementia as earlier diagnosis can also aid in the development of interventions that may reduce or delay the need for palliative care (Dorsey, Holloway, & Ravina, 2006). The strategy outlines the problems with current practice and recommends the development of new techniques in order to be able to achieve best recognition of early symptoms (Alzheimer's New Zealand, 2010).

Although biomarkers such as changes in the brain, blood and cerebrospinal spinal fluid in pre-symptomatic PD patients may facilitate earlier diagnoses of the disease in clinical settings, nothing yet exists to aid in discrimination of those patients most at risk for PD dementia. Additional biomarkers are still needed for greater accuracy in diagnosis of

the level of cognitive dysfunction (Shi, Bertrand, Hauber., & Zhang, 2009). Neuroimaging advances in recent years have shown that the distribution of pathology in neurodegenerative diseases affects the gross structural anatomy of some areas and directly contributes to the clinical presentation of disorders (Ashburner, et al., 2003). Examination of brain changes in neurodegenerative disorders therefore, may aid in improving diagnosis and monitoring of disease progression and provide a basis to test potential treatment options.

A transitional period of mild cognitive impairment (MCI) which falls between PD-N and PD-D has been recognised (Litvan, et al., 2011) and cited as a major risk factor for dementia in Parkinson's disease (Janvin, Larsen, Aarsland & Hugdahl, 2006). Work from our institute (Melzer, et al., 2011a) has shown that even in the very early stages of disease - in patients who exhibit cognitive function within the normal range (PD-N), there are significant cellular changes in the anterior cingulate, midbrain and corticospinal tracts. In PD patients with more impaired cognition (PD-MCI and PD-D) degeneration in these regions worsens and, in the anterior regions there is a significant association with cognitive status.

Neurological changes identified in PD-MCI could therefore aid in the future prediction of dementia (Aarsland, et al., 2003). Somewhat surprisingly, despite the early evidence for a rapid rate of conversion ~60% from MCI to dementia within four years in PD (Janvin, Aarsland, & Larsen 2005), the research strategies into the neurocorrelates of PD-MCI remain in their infancy. Investigation into the neurocorrelates of early stage Alzheimer's disease has been more forthcoming and indicates that imaging subcortical structures that are known to be involved in the later stage of disease may also provide insight into the pre-cognitive impairment period (Marek & Jennings, 2009).

1.3 Addressing a gap in the research: the thalamus and the progressive brain changes behind cognitive dysfunction

Although characterising PD-MCI in Parkinson's disease remains controversial, several recommendations have been made for suitable clinical criteria (Aarsland, et al., 2010; Dalrymple-Alford, et al., 2011; Jak, et al., 2009; Litvan, et al., 2011; Teng, Tingus, Lu, & Cummings, 2009), including recent formal guidelines (Litvan, et al., 2012). If these criteria are used in conjunction with neurological examination a more detailed picture of the characteristics of PD-MCI and the likelihood of progression to dementia could emerge.

Parkinson's disease is now recognised as a multi-faceted disorder that affects most cortical regions and disrupts multiple levels of cortical connectivity (Braak, Bohl, et al., 2006). Monitoring such widespread changes throughout the course of PD is beyond the scope of this thesis, but a representative approach was taken whereby the focus on the thalamus was examined, as this region reflects the functions of both the cortical mantle and the brain stem (Taber, Wen, Khan, & Hurley, 2004). The justification for this approach is clear. The central location of the thalamus means that all subcortical – cortical connectivity passes through this region (Behrens, et al., 2003; Cipolotti, et al., 2008); the neural connections of the thalamus are organised in a similar way to the architecture of the cortex, with anterior thalamic regions preferentially connecting to anterior regions of the cortex and, posterior thalamic regions preferentially connecting to the cerebellum and occipital regions (Jones, 2007b). Also, the thalamus reflects cortical degeneration as the level of thalamic atrophy often corresponds to the breakdown in cortical integrity. In patients with a frontal lobe tumours, for example, unilateral removal of the tumour resulted in ipsilateral retrograde atrophy of the thalamus in 9 of 12 patients (Hulshoff Pol, et al., 2000). More recently, the thalamus has demonstrated a strong relationship with cognition in several other neurodegenerative disorders (Byne, Hazlett, Buchsbaum, & Kemether, 2009; de Jong, et al., 2008; Houtchens, et al., 2007).

In Parkinson's disease, atrophy of the thalamus as a single structure is only evident in some cases (Lee, et al., 2011a) and the majority of samples show no thalamic changes relative to control subjects (McKeown, et al., 2008; Messina, et al., 2011; Peran, et al., 2010). Examining only the gross structure of the thalamus with no consideration of its components misses an opportunity to partial out the influence of the sub-regions of the thalamus on cognitive decline however. Parkinson's disease patients exhibit deficits in a range of cognitive functions, including attention, executive function, learning and memory and visuospatial dysfunction, sometimes in even the early stages before global cognitive dysfunction is evident (Janvin, Aarsland, Larsen, & Hughdahl, 2003). These sub-types of cognitive dysfunction have previously been attributed to specific thalamic regions (Kopelman, et al., 2001). Examination of the thalamus in this way could therefore provide a biomarker for the earliest identification of cognitive symptoms in PD and may be better than that achieved by examination of the thalamus as a single structure.

In the current thesis, quantative imaging analysis of standard structural and comprehensive DT images was employed. Volumetric changes and DTI were both utilised

to enable a multimodal investigation of macro and microscopic measures of local brain tissue damage and tructural thalamic disconnection. A cohort of Parkinson's disease patients who were classified on the basis of detailed cognitive testing was examined to establish the relationship between thalamic integrity and cognitive dysfunction. It was anticipated that thalamic volume and microstructural integrity would show a relationship with cognition and accurately discriminate between three levels of cognitive status in PD (PD-N, PD-MCI and PD-D). The current investigation is expected to clarify the importance of the thalamus in the pathophysiology of PD and PD-D and to provide a baseline for further follow up studies using a longitudinal design. To the best of our knowledge this will be the first study to encompass a cross-section of PD patients at various levels of cognitive status.

1.4 Objectives

1. Review neurological changes in PD (*Chapter 2*)
2. Review the relationship between the thalamus and cognition (*Chapter 3, Chapter 4*)
3. Investigate if thalamic change in PD is representative of cognitive decline: (Refer to individual study chapters outlined below)
4. Summarise findings (*Chapter 10*)

1.5 Hypotheses

1.5.1 That the thalamus will reflects cognitive dysfunction in Parkinson's disease

Study One: Structural magnetic resonance imaging

The primary objective of this study was to examine the influence of thalamic degeneration on cognition in Parkinson's disease using traditional region of interest methods. The thalamus was examined as a single brain region here, in contrast to later investigations which isolated sub-components of the thalamus. For the remainder of this thesis when the thalamus is examined as a single structure it will be referred to as an investigation of the 'whole thalamus'. Structural T1 imaging allowed for the examination of the whole thalamus and, for the first time, DTI, for the examination of subtler structural changes in the thalamus and their potential effect on cognition in PD.

The thalamus is involved in cognitive dysfunction in other neurodegenerative disorders (Batista, et al., 2012; Byne, et al., 2009; Coscia, et al., 2009; de Jong, et al., 2008; Houtchens, et al., 2007; Kassubek, Juengling, Ecker, & Landwehrmeyer, 2005; Seidenberg, et al., 2008; Stout, et al., 1999), and, by virtue of group comparisons between PD with and without dementia which have identified thalamic changes (Burton, McKeith, Burn, Williams, & O'Brien, 2004; Summerfield, et al., 2005), an association between the thalamus and cognition in PD has previously been established. Based on these findings we expected that if sensitive methodology was applied we would find that the thalamus was degenerated in those patients with dementia. Few studies (Dalaker, et al., 2011; Melzer, et al., 2011b) have included the thalamus for examination in PD-MCI and those that have only use whole brain voxel based methods. Neither of these studies identified thalamic degeneration in this group. Despite this we expected that some level of thalamic atrophy would also be evident in this group if stringent criteria were applied to identify this intermediate level of dysfunction.

We expected DTI measures to be more sensitive to cognitive dysfunction in PD as DTI allows for inference of cellular, rather than gross anatomical changes (Watts, 2008). Microstructural alterations in the absence of atrophy has been identified in the thalamus of one PD sample (Peran, et al., 2009) to date. Although these PD patients did not have dementia, they may have been exhibiting cognitive impairment as the cognitive examination of this sample was not sensitive to mild forms of impairment. We expect that microstructural alterations will be evident in our PD-MCI patient group and will worsen in our PD-D group.

Methodological and statistical limitations of previous research has not allowed for detailed examination of thalamic influence on cognitive dysfunction however. This is primarily due to small sample sizes and restricted neuropsychological testing (Messina, et al., 2011; Paviour, Price, Jahanshahi, Lees, & Fox, 2006a; Tinaz, Courtney, & Stern, 2010). In addition, in the majority of cases no examination of the cognitive state of the PD cohort has been conducted (Lisanby, et al., 1993; McKeown, et al., 2008; Peran, et al., 2009). We propose that when extensive neuropsychological testing is applied to a well characterised PD cohort and the motor confounds of PD taken into account that we will be able to demonstrate thalamic involvement in cognition.

Study Two: Voxel based morphometry

The primary objective of the second study was to examine the thalamus when it is mapped to standard space to validate the native space results as previous research has mainly applied standard space analysis. A second objective was to determine which areas within the thalamus were most subject to change, an objective that is not possible in native space.

We expected the thalamic degeneration identified in native space would also be present in standard space analysis. In line with previous research (Beyer & Aarsland, 2008; Summerfield, et al., 2005), and that of our own group (Melzer et al., 2011), we expect the progressive nature of cognitive decline within Parkinson's disease to correspond to grey matter reduction when the thalamus is isolated, similar to the progression of degeneration that is exhibited in the wider cortex (Beyer & Aarsland, 2008).

As voxel based morphometry (VBM) allowed for the examination of change within individual voxels of the thalamus we expected some regions to show degeneration while others did not. Two previous VBM studies have isolated regions within the thalamus as differentially affected in Parkinson's disease. Neither of these studies examined the effect region specific degeneration had on cognition however, with one focusing on neuropsychiatric (Cardoso, et al., 2009) and the other on the movement symptoms (Kassubek, Juengling, Hellwig, Spreer, & Lucking, 2002) of PD. Histological studies in Parkinson's disease do suggest degeneration is worse in some areas than others, especially in dorsomedial and ventral regions and that this relates to the level of global impairment (Halliday, 2009; Henderson, Carpenter, Cartwright, & Halliday, 2000a). We expect that the region which is most targeted by Lewy pathology (the non-specific centromedian/parafascicular region) will show the greatest change in our sample and have the strongest influence on Parkinson's symptoms, including cognitive dysfunction due to the connectivity of cortical regions known to be influenced by that nucleus.

1.5.2 That individual regions of the thalamus will be differentially involved in Parkinson's disease and reflect cognitive dysfunction.

Study Three: Diffusion tensor imaging investigation

Our third study employed advanced diffusion techniques (Wiegell, Tuch, Henrik, Larsson, & Wedeen, 2003) and examined the structural components of the thalamus and closely approximated anatomical guidelines of major thalamic nuclei. The primary aim of the

study was to investigate the association between each thalamic nucleus and cognitive dysfunction within the stratified PD cognitive groups (PD-N, PD-MCI and PD-D). As degeneration of the nuclei was hypothesised to be subtle we first had to determine the best imaging measure to capture this. Following investigation into the influence on cognition we then wanted to apply diffusion techniques to determine the nuclei which showed the greatest changes amongst the PD groups. One previous study (Li, et al., 2010) has investigated the diffusion measures of one thalamic nucleus in PD using a region of interest VBM approach of the whole thalamus but this study did not allow for comparison with volumetric measures, and was not conducted in regards to cognitive dysfunction.

In line with what has previously been established by others (Peran, et al., 2009) and ourselves (Melzer, et al., 2011a) in the first study we expected microstructural diffusion measures of the thalamic nuclei to be more reflective of cognitive diagnosis in PD than the volume of these nuclei. We hypothesised that diffusion measures would be more reflective of cognitive dysfunction in the thalamic nuclei than structural atrophy measures as this is true for the whole thalamus, and other subcortical structures in PD (Nicoletti, et al., 2006; Schocke, et al., 2004), and in other neurodegenerative disorders (Canu, et al., 2010).

We hypothesised that cellular or structural change in the thalamic nuclei would be associated with particular cognitive domains. Specifically, we hypothesised that the cognitive domain most associated with the nuclei's region of primary cortical connectivity would show the strongest relationship with that nucleus. We expected to see an association between the anterior principal (AP) and lateral dorsal (LD) nuclei and memory and executive function due to previous findings from lesion (Aggleton & Brown, 1999), histology (Byne, et al., 2006) and structural imaging (Gilbert, et al., 2001; Hazlett, et al., 1999) studies. We also expected the mediodorsal (MDn) to be implicated in executive function and memory due to the connectivity with prefrontal and frontal regions (Van der Werf, Witter, Uylings, & Jolles, 2000) and previous findings from Schizophrenia cohorts (Byne, et al., 2001). We expect an association with motor symptoms of PD and the centromedian/parafascicular (CM/Pf) complex as regions of the intralaminar nuclei have previously been implicated in PD-D (Henderson, Carpenter, Cartwright, & Halliday, 2000b). We also expect an association between this region and cognition due to the diffuse connectivity of this complex and the influence that the CM/Pf has over aspects of cognitive dysfunction in schizophrenia (Kemether, et al., 2003). We expected a significant association between the lateral posterior (LP) and pulvinar (Pu) and the

visuospatial/perception cognitive domain due to the previously established involvement of these regions in patients with thalamic lesions who exhibit significant visual deficits (Ricker & Millis, 1996) and, in regards to the pulvinar nucleus, possible involvement in working memory as this has previously shown to occur in other dementia samples (Andrews, Wang, Csernansky, Gado, & Barch, 2006; Harms, et al., 2007). In regards to the motor nuclei, ventral anterior (VA) and ventral lateral (VL), and the sensory ventral posterior (VP) nucleus we did not expect a relationship with any domain of cognition.

Due to the prominent connectivity with the regions of the cortex (Behrens, et al., 2003) that show the first signs of alterations in PD (Gattellaro, et al., 2009; Karagulle Kendi, Lehericy, Luciana, Ugurbil, & Tuite, 2008), we expect that the limbic nuclei will show microstructural alterations in the PD groups who have neither PD-D or PD-MCI. Frontal and temporal areas show the most degeneration in PD-D, with the frontal regions especially affected as early as PD-MCI (Beyer, Janvin, Larsen, & Aarsland, 2007). Thus, we hypothesise that the nuclei showing primary connectivity with these regions will be the first to show degeneration in our sample. Specifically, we expect the limbic nuclei to become implicated in the PD-N group who have no cognitive dysfunction or in the PD-MCI group who have intermediate cognitive dysfunction and the association (MDn) nuclei and possibly also the CM/Pf complex implicated in the PD-MCI group. The remaining nuclei are not expected to show degeneration until PD-D.

1.5.3 That cortical connectivity will be disrupted between the thalamus and the cortex in Parkinson's disease and contribute to cognitive symptoms

Study Four: Tractography Investigation

Our final study extended the diffusion study of the thalamic nuclei and examined the integrity of the major cortical connections between each thalamic sub-region and the cortex. Tractography is a new diffusion tensor technique that allows for the quantitative examination of the integrity of any major subcortical-cortical connections (Hasan, Kamali, & Kramer, 2009). Previously, the relationship between cognitive or motor symptoms in Parkinson's disease and connectivity impairment has only been able to be hypothesised based on other factors such as cell loss or atrophy of subcortical regions. To date, only our own group (Melzer, et al., 2011a) has specifically set out to isolate white matter pathways in Parkinson's disease and determine the influence of these on cognition.

In line with what we expect from the thalamic nuclei, we also expect the fibre tracts originating from the thalamus to have an association with cognitive dysfunction in Parkinson's disease. The white matter pathways in the mouse model of Parkinson's disease are thought to be the main facilitators of Lewy body distribution (Luk, et al., 2012) and thus we expect them to be significantly disrupted. As Lewy distribution worsens alongside cognitive dysfunction (Braak, Rub, & del Tredici, 2006) we expect this to be reflected in the fibre tracts.

1.6 Summary

There is an urgent need to identify the early stages of cognitive decline in Parkinson's disease due to the rapidly increasing aging population (Statistics New Zealand 2008) and the corresponding increase in dementia development that this brings (Janvin, et al., 2006). The thalamus is a potential neurocorrelate of cognitive dysfunction in Parkinson's disease due to the influence on several behaviours and processes (Van der Werf, et al., 2000) known to be affected in this disorder and also shows a relationship with cognitive decline in other neurodegenerative disorders (de Jong, et al., 2008; Houtchens, et al., 2007). This thesis addresses the relationship between thalamic degeneration and cognitive decline in Parkinson's disease across four studies. We expect the thalamus to have a relationship with cognition, for there to be differential involvement of thalamic regions with the limbic nuclei to be most affected and, finally for there to be a corresponding decrease in the integrity of the thalamic fibre tracts.

Chapter 2. THE NEUROLOGICAL CHANGES IN PARKINSON'S DISEASE

2.1 Objectives

This chapter explores neurological changes in Parkinson's disease with a particular focus on the neurotransmitter and neuropathological correlates of movement, neuropsychiatric and cognitive symptoms. The majority of the neuropathology research has been post-mortem, but where applicable *in vivo* studies are also discussed here, although structural (*Chapter 6; Chapter 7*) and cellular changes (*Chapter 8;Chapter 9*) are addressed in the relevant study chapters.

2.2 Neurological changes in PD

Like Alzheimer's disease, the damage in the PD brain accumulates over time and the observable symptoms are the end result of a cumulative pathological process that is not identifiable clinically until the disease has reached an advanced stage (Braak, et al., 2000). The first symptoms are the result of the degeneration of dopaminergic cells in the substantia nigra, which begins before symptoms are present, and at autopsy has cumulated in a cell loss of 50-70% (Braak, Bohl, et al., 2006). The majority of neurological changes in PD directly affect, or are mediated by the thalamus. The thalamus is a component of the extrapyramidal system, for example which controls movement (Brooks, et al., 1990), the frontal-striatal system which is involved in neuropsychiatric symptoms of PD (Murai, et al., 2001) and the limbic system which is significantly affected in PD and correlates with cognitive dysfunction (Kaasinen & Rinne, 2002). Although dopaminergic changes are traditionally considered to be the hallmark of PD, the interaction between many different neurochemicals and the neuropathology in several cortical regions contributes to the multiple facets of this disorder.

2.2.1 Lewy pathology

The main pathological feature of PD is the presence of Lewy bodies (LB) and Lewy neurite (LN) inclusions that are formed after the alpha-synuclein (α-sync) protein becomes misfolded (Rub, Del Tredici, Schultz, et al., 2002). Normally the α-sync protein only accounts for 1% of the proteins in the brain and plays a role in the modulation of synaptic transmission, synaptic vesicle density and neuronal plasticity (Abeliovich, et al., 2000; Murphy, Rueter,

Trojanowski, & Lee, 2000). It has also been suggested that α-sync has a role in neurogenesis (Crews, et al., 2008) and cell survival (Yasuda & Mochizuki, 2010). Although the underlying cause is unknown (Braak, Tredici, Rub, de Vos, Jansen Steur & Braak, 2003), when α-sync becomes misfolded the aggregations that form, along with other neurofilaments and synaptic vesicle proteins generate the characteristic Lewy bodies found in the cytoplasm of neurons in PD patients.

2.2.1.1 The quantification of disease stage

The spread and degree of Lewy inclusions in the Parkinson's brain have recently been used to develop a scheme of disease stage (*Table 2-1*). These inclusions begin developing before symptoms are present and spread throughout the cortex in six distinct stages, eventually infiltrating all cerebral areas (*Figure 2-1*).

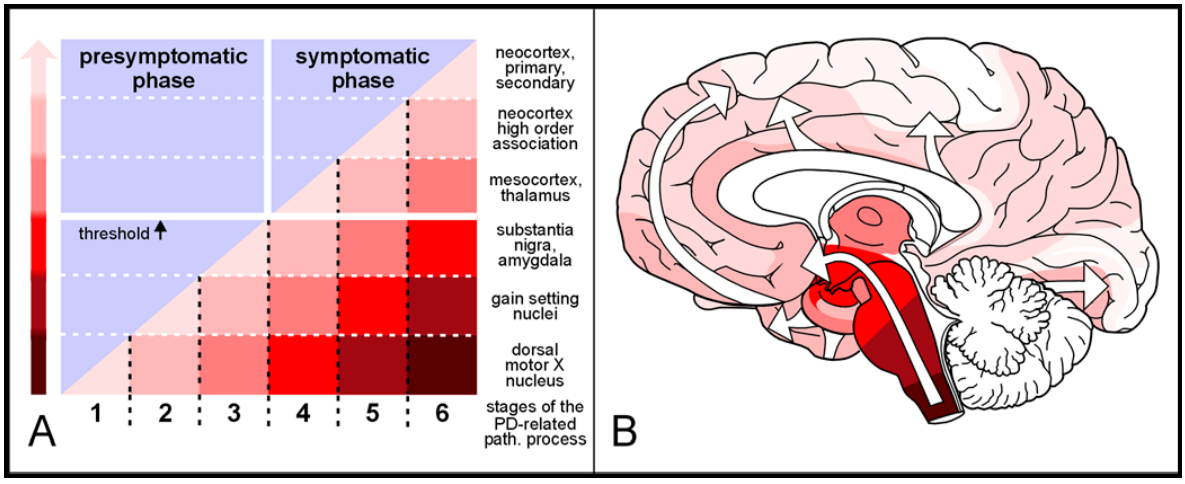


Figure 2-1: Braak's (2004) stages of disease progression
A: The thalamus is implicated in stage 3, Lewy neurites and bodies are evident in brains of asymptomatic patients, once the threshold is exceeded however symptoms worsen with increasing levels of pathology. **B:** Pathology begins in lower cortical regions and proceeds caudally until all regions of the cortex are affected.

Lewy body pathology is at first confined to the medulla and moves from here in a dorsal direction to the pons, reaching the midbrain in stage three and the mesocortex in stage four before finally encompassing the neocortex in the final two stages (Braak, et al., 2003). This model of pathology progression is most applicable to those with younger onset PD who have a long disease duration as between 6.3 and 43% of cases followed a different pattern of Lewy progression, and, when the criteria has been applied to larger samples 49% of cases were unable to be classified according to this criteria (Jellinger, 2008). Only some nuclei of the thalamus show Lewy inclusions. According Braak's (2002) model of progression the motor VA and non-specific intralaminar nuclei are the only regions to show Lewy pathology.

Other histological studies in PD that have focussed on the thalamus confirm that Lewy inclusions are evident in these areas as well as in the AP and MDn nuclei of the limbic loop, and that all regions are similarly affected by neuronal and volume loss. The mediodorsal and ventral anterior nuclei of the thalamus are most affected by Lewy pathology when the limbic loop becomes affected (Halliday, Macdonald, & Henderson, 2005).

Table 2-1: Braak's stages of disease progression in PD

Stage		Main Areas Affected
1		Dorsal motor nucleus Anterior olfactory structures
2		Portions of lower Raphe nuclei Portions of reticular formation Locus coeruleus
3	Manifestation of motor symptoms	Pars compacta of Substantia Nigra Magnocellular nuclei Central subnucleus of amygdala Cholinergic axons from basal forebrain that pass through external capsule
4		Amygdala Ammon's horn Temporal mesocortex (incl. olfactory cortex and hippocampus)
5	Cognitive decline evident when tested with MMSE	Paling of substantia nigra Projections into sensory association areas Anterior cingulate cortex Prefrontal areas
6	Correlation between degeneration and cognition seen in final stages	First order sensory association Premotor areas Primary motor field Primary sensory association Involvement of entire neocortex

The progression of Lewy body pathology and association with Parkinson disease symptoms as outlined by Braak (2006).

Lewy pathology itself is not detectable using neuroimaging techniques (Vernon, Ballard, & Modo) but it is now possible to image amyloid load using contrast agents in MRI (Klunk, et al., 2003). As Lewy pathology precedes neuronal loss (Dickson, Uchikado, Fujishiro, & Tsuboi, 2010) and neuronal loss to a large degree is reflected in gross atrophy of cortical regions (Halliday, 2009), MRI of atrophy in the neurodegenerative brain could reflect clinical symptoms related to Lewy progression.

Neuropathology at autopsy is the only way to definitively diagnose PD, but the distinct early neurological changes suggest pre-symptomatic diagnosis is a future possibility (Olanow & Obeso, 2012). Although the cause of these early neurological changes is not clear, the

progression of neurochemical and pathological changes in the PD brain are relatively well documented and generally allow for adequate monitoring of clinical symptoms, especially in relation to movement dysfunction.

2.2.2 Neurochemical changes

The neurotransmitter most involved in PD is dopamine (DA) and the main source of straital dopamine is from the pars reticular component of the substantia nigra (SNr). Although theories behind the initial postulation of DA loss are abundant, the only unequivocal risk factor for increasing cell loss is advancing age. Advancing age is a risk factor for Parkinson's disease, but PD does not manifest as part of the normal aging process. DA neuronal loss in PD is severe and involves different subpopulations of the substantia nigra pars compacta (SNc) (Bezzard & Gross, 1998). In normal aging, for example, the dorsal tier of the substantia nigra is lost but in PD it is the ventral tier that is most affected (Gibb & Lees, 1991) and there remains no clear evidence that PD is an exaggeration of the ageing process (Rakshi, et al., 1999). The degeneration of dopaminergic neurons and their projections may take decades to develop and motor signs appear only after 50 - 70% of neurons are lost (Jellinger, 1999). The loss of dopamine in extrapyrimidal parts of the motor loop contributes to most of, if not all observable symptoms of the disorder.

2.2.2.1 Movement Symptoms

Dopaminergic cells normally serve to mediate motor function through the release of dopamine into the extrapyramidal system. The first visible symptom of Parkinson's disease is of movement dysfunction such as tremor at rest, postural instability and difficulty in executing movement (Hughes, Daniel, Kilford & Lees, 1992). In most cases motor dysfunction initially affects only one half of the body, gradually progressing to the contralateral side. Bradykinesia, slowness in the execution of movement is a key feature of the disorder. Patients have difficulty dressing, show micrographia, slower walking speed and quieter speech. Patients will also have at least two of the following symptoms. Resting tremor is common and will abate with action or in sleep and almost always appears in the distal part of an extremity. Rigidity in passive movement is characterised by increased resistance in muscle tone when moving and may also be associated with the presence of pain. The cogwheel phenomenon, when pushing on an arm causes it to move in jerky increments instead of smoothly, usually accompanies symptoms of rigidity. Postural instability, the loss

of postural reflexes, is generally a manifestation of the late stages of the disease, occurring after onset of other clinical features (Jankovic, 2008). The neurological changes behind movement dysfunction are perhaps the most well researched phenomenon in PD but the precise reasons for the origin of symptoms are still beyond our grasp.

Dopaminergic cells primarily serve the motor loop which originates in the motor area of the cortex and terminates in the striatum portion of the basal ganglia. The striatum is the main region to receive dopaminergic projections from the substantia nigra so DA reduction directly disrupts the normal process of the motor loop and other downstream dopaminergic processes (Obeso, Rodriguez-Oroz, Rodriguez, DeLong, & Olanow, 2000). From the striatum, neurons project back to the cortex via either the direct or indirect pathway. The final output of the direct pathway is excitatory and stimulates the motor cortex and results in a movement increase. The final output of the indirect pathway is inhibitory which dampens the motor cortex and results in a movement decrease. *Figure 2-2* depicts the normal functioning of the motor loop (left) and the disrupted loop in Parkinson's disease (right). The direct pathway causes a net movement increase through inhibition of the SNr-globus pallidus-internal (GPi) complex. Because projections from this region normally act to inhibit the thalamus, when the action of the SNr-GPi complex is reduced they no longer inhibit thalamic activity and the motor nuclei of the thalamus (VA and VL), stimulate the cortex to facilitate an increase in movement. In contrast, the normal function of the indirect pathway is to send inhibitory messages to the external portion of the globus pallidus (GPe), eventually causing reduced output from the thalamus. In Parkinson's disease, the cortico-striatal balance is disrupted, leading to reduced activity in the direct circuit and increased activity in the indirect circuit and excessive inhibitory output of the GPi/SNr. The thalamic components of this system do not show a large degree of pathology or neuronal loss, instead the intralaminar regions, and, to a lesser degree, the limbic regions of the thalamus are implicated in PD (Henderson, et al., 2000b).

Dopaminergic neuronal death continues after the first appearance of clinical symptoms, resulting in significantly decreased dopamine levels throughout the cortex and further increase in motor disturbances. Although dopamine therapy can initially correct movement imbalances it leads to a highly unstable system and departure from the normal physiological basal-ganglia system (Olanow, Lees, & Obeso, 2008). Dopamine dysfunction in PD therefore has long reaching consequences that are not easily fixed.

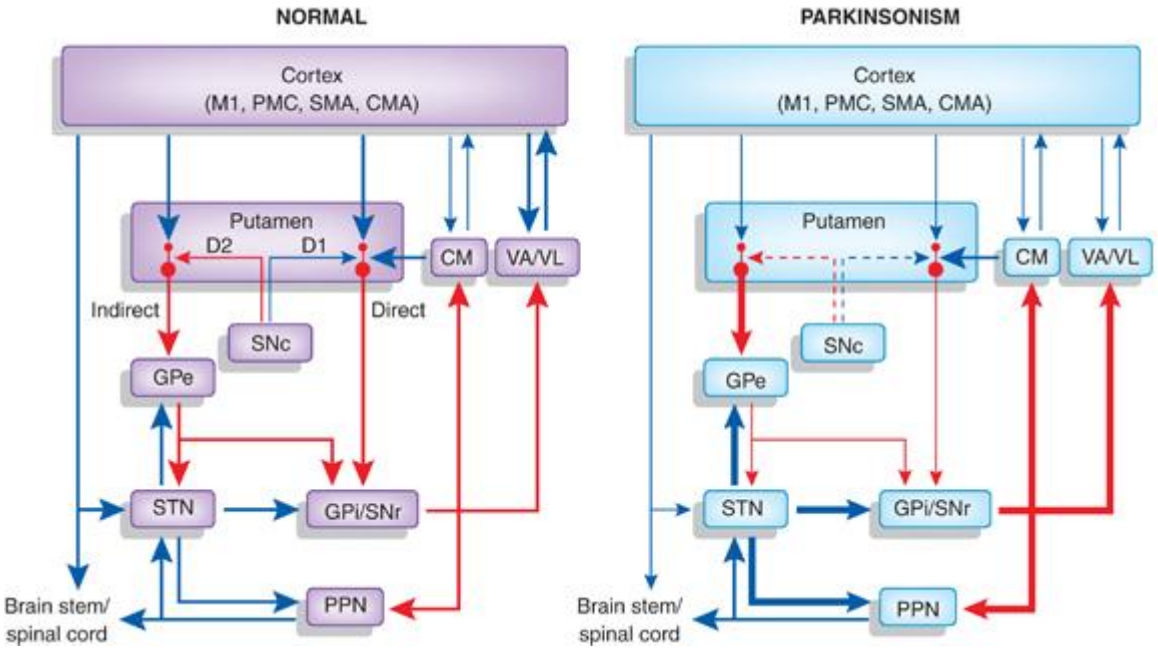


Figure 2-2: The role of dopamine in the normal and Parkinsonism brain

Schematic diagram from Smith, Wichmann, Factor & DeLong (2012) shows the direct and indirect pathways of the basal ganglia motor circuits in the normal (left) and PD (right) brain. Red arrows are inhibitory projections and blue arrows excitatory. The thicknesses of the arrows indicate the increase (larger) or decrease (thinner) in the activity of connections. Dashed arrows indicate the partial lesion of that connection in PD. **CM:** centromedian nucleus; **CMA:** cingulate motor area; **GPe:** globus pallidus, external segment; **GPi:** globus pallidus, internal segment; **M1:** primary motor cortex; **PMC:** pre-motor cortex; **PPN:** pedunculopontine nucleus; **SMA:** supplementary motor area; **SNc:** substantia nigra pars compacta; **SNr:** substantia nigra pars reticulata; **STN:** subthalamic nucleus; **VA/VL:** ventral anterior/ventral lateral nucleus. The motor nuclei of the thalamus (VA/VL) are most involved in this loop but the CM/Pf is the region to show the largest degree of Lewy degeneration.

2.2.2.2 Neuropsychiatric Symptoms

In addition to motor dysfunction there are a number of non-motor complications in PD which are also mainly influenced by dopamine dysfunction (Davie, 2008). In a comprehensive study of 150 patients with Parkinson's' disease, 16% of patients had one neuropsychiatric symptom assessed by the neuropsychiatric inventory (Cummings, et al., 1994), 28% of patients had two symptoms and 25% had three or more symptoms. The most common neuropsychiatric symptoms were depression (38% of patients), hallucinations (27%) and anxiety (20%) (Aarsland, Larsen, Lim, et al., 1999). Symptoms mediated by dopamine dysfunction increased with more advanced disease and all (delusions, hallucinations and agitation) were significantly correlated with Hoehn and Yahr stage and degree of cognitive dysfunction (Mancini, et al., 2004).

Depression is related to dopamine and noradrenalin levels. Depressed PD patients have lower chemical levels in the locus coeruleus and regions of the limbic system (anterior cingulate cortex; thalamus; amygdala and ventral striatum) (Poewe & Luginger, 1999).

Depression is more common in those with prominent bradykinesia and gait instability than those with tremor-dominant syndromes (Cummings, 1992) and it is not surprising that even sub threshold mood fluctuations that are common in advanced stages of the disease are related to levels of motor dysfunction (Richard, et al., 2004) and can be alleviated with dopamine therapy alongside selective serotonin reuptake inhibitors (Burn, 2002).

Psychosis is also mediated by dopamine. There is severe depletion of dopaminergic neurons in the mesolimbic areas in both PD with dementia and dementia with Lewy bodies (Naimark, Jackson, Rockwell, & Jeste, 1996). Visual hallucinations are also mediated by dopaminergic and cholinergic systems (Papapetropoulos & Mash, 2005) and functional imaging shows several abnormalities in the activity of associative areas in PD patients with visual hallucinations. Increased activation in the superior and inferior fontal gyrus (Stebbins, et al., 2004) and hypermetabolism in the frontal gyrus (Nagano-Saito, et al., 2004) are common findings and suggest significant disruption in the dopaminergic system.

2.3 The influence on cognitive dysfunction

Although James Parkinson noted that the movement disease which was later to be named after him left the 'senses and intellects uninjured' (Chapter 1, pg.1. Parkinson, 1817) the current understanding in PD is that cognitive impairment can occur even in the very early stages of diagnosis (Muslimovic, Post, Speelman, & Schmand, 2005) and, for the majority of individuals cumulates in dementia in the final years (Aarsland, et al., 2003). Cognitive dysfunction is perhaps now the most widely recognised non-motor complication of Parkinson's disease (Chaudhuri, Healy, & Schapira, 2006). Mediated by multiple brain regions and neurotransmitter symptoms, cognitive decline presents a heterogeneous profile in PD patients (Janvin, et al., 2003), especially in the early stages (Muslimovic, et al., 2005). It has long been accepted that dementia is a regular occurrence in Parkinson's disease. The cumulative prevalence of PD-D is 80% (Aarsland, et al., 2003) and will manifest around 10 years after Parkinson's disease is first diagnosed (Hely, Reid, Adena, Halliday, & Morris, 2008; Hughes, et al., 2000; Williams-Gray, Foltynie, Brayne, Robbins, & Barker, 2007). In more recent years, the prevalence of mild cognitive impairment has also been a prominent discussion in Parkinson's disease research. Although there is a wide range of applied criteria available (Aarsland, et al., 2010; Janvin, et al., 2006; Petersen, et al., 1999), including our own (Dalrymple-Alford, et al., 2011) to diagnose MCI in PD it is generally accepted that it is

a transitional stage occurring intermediary to no cognitive impairment in early PD and dementia in late PD.

2.3.1 Dementia

Dementia is characterised by a decline from normal cognitive functioning and is accompanied by an inability to retain previously well established social and behavioural skills. Patients display a significant decline in daily functioning and exhibit fluctuating impairment in attention, deficits in most aspects of executive functions, visuospatial deficits which are disproportionate to the severity of dementia and will also have an impairment in retrieval memory, although this is generally mild (Emre, 2010). The decline to dementia is progressive, risk significantly increases as PD advances (Hely, Morris, Reid, & Trafficante, 2005).

2.3.2 Mild Cognitive Impairment

PD patients must experience a transitional period of impairment prior to the onset of dementia. Point - prevalence estimates range from 22-55%, depending on the criteria used for diagnosis (Janvin, et al., 2003; Verbaan, et al., 2007). PD-MCI is characterised by a decline in function but without the accompanying difficulty with everyday tasks (Litvan, et al., 2012). That is, PD-MCI patients experience memory problems or attention deficits (Aarsland, et al., 2010) but will continue to engage in activities such as shopping and organising finances (Reisberg, et al., 2001). Areas of cognitive impairment are the same in PD-MCI as those affected in PD-dementia, executive function, working memory, visuospatial function and attention difficulties, but generally at a lower level than in PD-D (Emre, 2010). A number of cognitive domains can be affected, but any single domain impairment without accompanying memory deficit is the most common form of MCI in PD (Litvan, et al., 2011).

Because not all PD patients develop dementia while others have early onset dementia, this suggests there are underlying factors which influence the likelihood of dementia development. Identifying precursors to dementia would aid in recognition of those most at risk and could even aid in early intervention for pre-symptomatic patients. The risk factors for early onset dementia include: older age, severity of motor symptoms (Alves, Larsen, Emre, Wentzel-Larsen, & Aarsland, 2006; Burn, et al., 2006; Williams-Gray, et al., 2007), visual hallucinations (Aarsland, et al., 2003; Aarsland, et al., 2004) and mild cognitive impairment (Janvin, et al., 2006). Mild cognitive impairment, especially when consisting of

executive function and memory deficits is the biggest risk factor to the later development of dementia (Janvin, et al., 2005). A better understanding of this transitional phase will enable identification of those patients at highest risk for dementia and aid in employing interventions to delay or prevent further cognitive decline.

2.4 Pathology

The neurological changes in PD include both PD-pathology (Lewy body accumulation) and AD-pathology (amyloid deposits and neurofibrillary tangles). Amyloid pathology has a linear relationship with cognition, exhibiting lowest levels in controls and the highest in PD-D with the level of pathology in PD patients without dementia at an intermediate level (Compta, et al., 2009). The increasing severity of lesion burden has a direct association with dementia in the final three Braak stages (Braak, Rub, et al., 2006).

The gradual accumulation of Lewy bodies, especially in subcortical structures is thought to induce mild cognitive impairment and finally dementia in Braak stages 5-6 of PD patients (Braak, Rub, et al., 2006). Because LB burden in the PD brain is not uniformly distributed, the level of burden is not as important as the location, with the pattern of α-synuclein pathology thought to be more influential over the development of PD symptoms than the level of pathology. Specifically, it is LB pathology in the limbic system that appears to have the biggest association with dementia (Kovari, et al., 2003). *Figure 2-3* shows that in stage 3, when the limbic system first becomes infiltrated is also the stage where patients transition from the cognitive pre-symptomatic to cognitive symptomatic stage (Braak, Bohl, et al., 2006) of impairment. In terms of the thalamus, the anterior and mediodorsal regions become involved as early as stages 3 and 4 respectively and are therefore likely to play a significant role in the transition to PD dementia. Although there appears to be an association between the locality and degree of degeneration and PD symptoms, the type of clinical symptoms that are influenced by this pathology still mostly remains unclear (Emre, 2010).

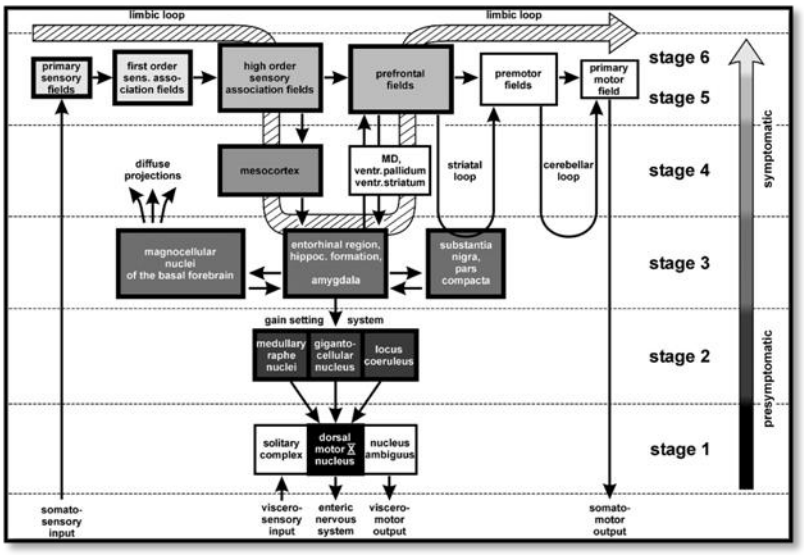


Figure 2-3: The pattern of Lewy pathology burden in PD
The limbic sites that are crucial to learning and memory become involved at stage 4 and contribute to the development of cognitive decline. The components of the limbic loop are essential to the transfer of information from the association cortex to the prefrontal cortex via regions of the basal ganglia and the mediodorsal thalamus. Image from Braak, Bohl, et al., (2006).

The location of Lewy inclusions appears to be a significant factor in dementia development as LB density is greatest in the limbic system (entorhinal cortex and anterior cingulate cortex) and has the strongest relationship with cognition in PD (Kovari, et al., 2003). Lewy bodies in the frontal gyrus especially have been found to be the most significant predictor of cognitive impairment in a PD sample (Mattila, Rinne, Helenius, Dickson, & Roytta, 2000). In Parkinson's disease with dementia Lewy body distribution was found to be highly sensitive (91%) and specific (90%) markers of dementia (Hurtig, et al., 2000).

Lewy pathology is related to cortical atrophy levels, a greater number of Lewy bodies contribute to a larger degree of cortical volume loss. In PD patients with late onset PD and patients with diffuse Lewy body disease for example, there is a significant degree of atrophy in the frontal lobes at death. There was no difference in the level of atrophy in the DLB disease patients compared to the late-onset PD patients, indicating that regardless of the underlying cause, LB pathology can induce volume loss in neurodegenerative disorders (Double, et al., 1996).

Braak's (2006) model of disease progression stipulates that pathology begins in the brain stem and works steadily upward, increasing the degree and type of symptoms. This model fits with the clinical presentation of cognitive symptoms in PD (Janvin, et al., 2003) and has been verified with neuroimaging. Degeneration occurs in the brainstem regions prior to the development of cognitive symptoms (Jubault, et al., 2009), progressing into the limbic

regions as cognitive dysfunction becomes apparent (Beyer, Janvin, et al., 2007) and finally infiltrating the remaining neocortex in those patients with PD-D.

Current MRI protocols do not allow for the study of burden in the thalamus but volumetric differences and the disruption in cellular architecture within the thalamus can be examined using traditional and advanced MR and DT imaging techniques (Wolf, et al., 2003). Both of these techniques can be considered to be influenced by lesion burden as the accumulation of Lewy bodies in neurons has been shown to cause cell death, atrophy and lead to the clinical manifestation of symptoms (Cordato, Halliday, Harding, Hely, & Morris, 2000). MR imaging allows for examination of any gross structural abnormalities of the thalamus, where volume loss is assumed to represent the cumulative degeneration of neurons and supporting cells and the loss of extracellular space (McKeown, et al., 2008). DT imaging allows for more subtle analysis of changes at the microstructural level and is assumed to represent the degeneration of axons and damage of cells within a structure (Rose, Janke, & Chalk, 2008)

2.5 Summary

PD is a neurodegenerative process which affects multiple systems and arises from dopamine depletion in the substantia nigra. Lewy body pathology is the hallmark of Parkinson's disease but this is best identified at autopsy (Braak, Bohl, et al., 2006). Pathology of this type does induce neuronal loss however which manifests as atrophy in some cases and can be detected using neuroimaging techniques (Beyer, Larsen, & Aarsland, 2007; Double, et al., 1996; Song, et al., 2011). Although movement dysfunction is the hallmark of Parkinson's disease, pathology has also been shown to relate to neuropsychiatric and cognitive symptoms. Changes in thalamic mediated systems underlie these symptoms and *in vivo* identification of thalamic abnormalities in PD could be a useful neurocorrelate of dysfunction. Cognitive dysfunction is especially relevant as the progression to dementia could be monitored or predicted through regular neurological examination. Given the involvement of the thalamus in multiple systems which mediate the main symptoms of PD we hope that detailed examination of the thalamus will provide an adequate neurocorrelate of dysfunction.

Chapter 3. THE THALAMUS

The thalamus is centrally located and reciprocally connected with multiple areas of the cortex, influencing a wide range of functions and behaviours (Taber, et al., 2004). The location and connectivity the thalamus has with the wider cortex means it is grossly affected in neurodegenerative disorders and thalamic degeneration has a strong relationship with both the clinical and cognitive symptoms of multiple diseases. In Alzheimer's disease dementia (AD) for example, the volume of the thalamus is significantly reduced (de Jong, et al., 2008), a phenomenon that has also been observed in Huntington's disease (Kassubek, et al., 2005), multiple sclerosis (Houtchens, et al., 2007), schizophrenia (Brickman, et al., 2004; Gilbert, et al., 2001; Qiu, Zhong, Graham, Chia, & Sim, 2009) and temporal lobe epilepsy (Seidenberg, et al., 2008; Stewart, et al., 2009). In the majority of these disorders the degree to which the thalamus is reduced reflects the severity of cognitive dysfunction.

Thalamic changes have also been identified at the cellular level and the extent of microstructural disruption shows a relationship with the severity of symptoms. In Alzheimer's disease (Rose, McMahon, et al., 2006), the microstructure of the thalamus is significantly altered and has a strong relationship with multiple aspects of cognition – especially with measures of executive function. Microstructural changes in this disorder are also strongly correlated with the level of volume loss. The identification of microstructural changes is especially useful as, prior to meeting criteria for AD the majority of patients will first enter a transitional phase of mild cognitive impairment (Apostolova, et al., 2006). In patients at the MCI stage, a disruption in cellular integrity was identified and had a relationship with cognitive dysfunction despite thalamic volume being maintained (Cherubini, et al., 2010).

In Parkinson's disease (PD), research on thalamic involvement is limited and has mainly been conducted using neuro-histology methods (Halliday, 2009; Henderson, et al., 2000a). Where MR methods have been applied, the transitional PD-MCI stage has generally been ignored (McKeown, et al., 2008) or the thalamus has only been examined using a standard template (Beyer, Janvin, et al., 2007), a practice known to warp the true nature of degeneration, especially in a sample of older participants (Mechelli, Price, Friston, & Ashburner, 2005).

3.1 Objectives

The aim of this review chapter is to first provide a background on the normal function of the thalamus in order to be able to understand subsequent chapters where thalamic degeneration in Parkinson's disease is explored. In order to comprehend how thalamic changes may affect normal processes, the effect of thalamic degeneration in other neurodegenerative disorders is also discussed. The thalamic changes that occur in Parkinson's disease will be explored in subsequent chapters that employ the same methodology as these previous studies. The methodology that has been used to investigate the thalamus in PD thus far includes structural imaging in both native (McKeown, et al., 2008) and standard space (Beyer, Janvin, et al., 2007) as well as diffusion tensor imaging (Cherubini, et al., 2010) and thalamic changes in PD that have been identified using these techniques will be reviewed in chapters *Chapter 6*, *Chapter 7*, and *Chapter 9* respectively.

3.2 The role of the thalamus in cognition

Although the thalamus has traditionally been considered a 'relay system,' which receives messages from the sensory system and relays them to the appropriate cortical area, there is now significant evidence which suggests that the thalamus is also independently involved in multiple aspects of functioning (Sherman, 2007). Information gained from individuals who have suffered localised thalamic strokes, or other forms of brain lesions, for example provides strong evidence that the thalamus contributes, rather than merely facilitates cognitive function (Carrera & Bogousslavsky, 2006; Van der Werf, et al., 2003; Van der Werf, et al., 2002). In order to understand the significance of thalamic damage however we must first examine the normally functioning thalamus.

3.2.1 Location and composition

The thalamus is a large aggregation of nuclei that is situated in the diencephalon – one of the major subdivisions of the brain that lies between the forebrain and midbrain (Jones, 2007a). As the largest component of the limbic system, the thalamus is easily identifiable in MR images. Although it is surrounded by the other grey matter structures of the basal ganglia, the immediate borders of the thalamus include the white matter of the external capsule and, medially, the third ventricle which provide a great contrast in MR images (*Figure 3-1*).

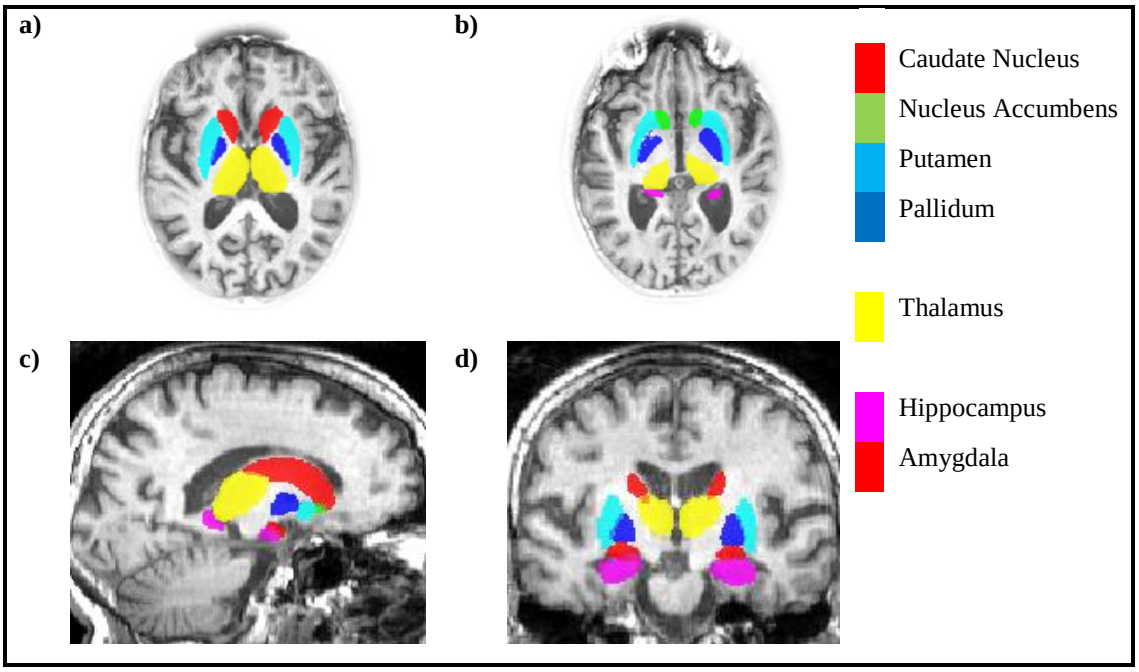


Figure 3-1: FIRST segmentation of the basal ganglia
Horizontal (top) sections at the **a)** dorsal (Z = 100) and **b)** ventral (Z = 92) level show the thalamus surrounded by the components of the basal ganglia. The thalamus is easily identifiable in MR images due to being surrounded by the lateral (**c:** saggital section, X = 118) and third (**d:** coronal section Y = 22) ventricles. Co-ordinates are in MNI space.

3.2.2 Connectivity

Nearly all incoming sensory information is relayed through the thalamus before being transmitted to wider cortical areas – a system which underlies many cognitive, behavioural and motor functions (Sherman & Guillery, 2004). Information is relayed through a series of fibre connections, each beginning, and ending in a specific location of the cortex (*Figure 3-2*).

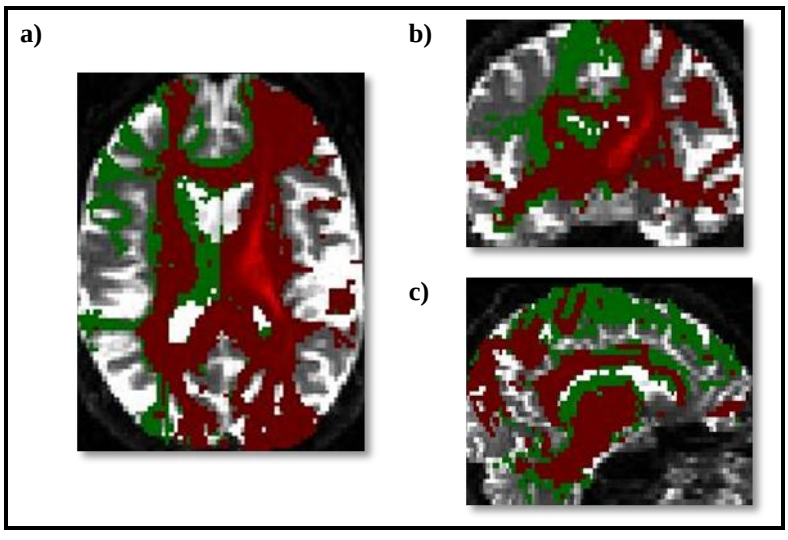


Figure 3-2: Thalamic connectivity
a) Horizontal Z = 58, **b)** coronal Y = 66 and **c)** saggital Z = 29 sections of the thalamic fibre tracts that originate in left (red) and right (green) thalamus.

There are three main areas of the thalamus which receive and send fibre connections to specific areas of the cortex: relay nuclei; intrinsic nuclei and the midline & intralaminar nuclei. The relay nuclei receive input from the basal ganglia, limbic system, cerebellum, brain stem and spinal cord and transfer information to the motor and limbic regions of the cortex – influencing movement and cognition. The intrinsic nuclei receive projections from within the thalamus and project to the association areas of the cortex, regions within the occipital, parietal and motor cortices and serve to mediate functions such as vision, hearing, touch, temperature, and the interpretation of pain and taste. The midline and intralaminar nuclei receive input from multiple lower sources including the brain stem, spinal cord and the cerebellum and project fibres to the basal ganglia, and to a lesser degree also to the somatosensory, motor and premotor cortices which influences multiple movement and cognitive processes (Aglioti, 1997). These three functional groups of thalamic nuclei can be further subdivided into individual nuclei, each of which project to smaller areas within the cortex and the differential connectivity of which will be explored in the subsequent chapter (*Chapter 4*).

3.2.3 Function

The thalamus is a crucial component for multiple functions, including cognition and movement (Jones, 2007a), with the only function not mediated by the thalamus thought to be olfactory processing (Wilson & Mainen, 2006). The fibre networks that enable these processes to be carried out depend on the intact structure of the thalamus, as nearly all of these fibres traverse at least one thalamic region (Behrens, et al., 2003). The fibres are preferential, with those that mediate each processes only passing through a specific thalamic region. Each region within the thalamus therefore mainly contains neurons that preferentially connect to only one or two cortical areas. In the case of motor function, for example, the motor nuclei of the thalamus will mainly consist of motor projection neurons which primarily terminate in the motor cortex (Jones, 2007a).

There are two functionally distinct types of thalamic nuclei. First order relay nuclei carry messages from the lower brain to the neocortex and include the: anterior thalamic nucleus; the ventral anterior; ventral lateral; and the ventral posterior thalamic nuclei. Higher order relay nuclei receive incoming messages from the cortex itself and relay these messages from one cortical area to another. Nuclei included in this division are the: mediodorsal nucleus which links one part of the frontal cortex to another; the laterodorsal nucleus which

links two areas of the cingulate cortex together; the pulvinar complex and the lateral posterior nuclei, both of which link areas of the occipital and temporal lobes together; and the intralaminar nuclei which span both first order and higher order relay nuclei divisions – linking lower brain regions with the neocortex while also linking areas of the motor cortex to the striatum (Sherman & Guillery, 2004). The communication between these areas is possible due to the chemical transmission of signals between the cells, or neurons in each area. Each neuron (*Figure 3-3*), although there are several different types, consists of a cell body, dendrites, an axon and axon terminals (Stufflebeam, 2008).

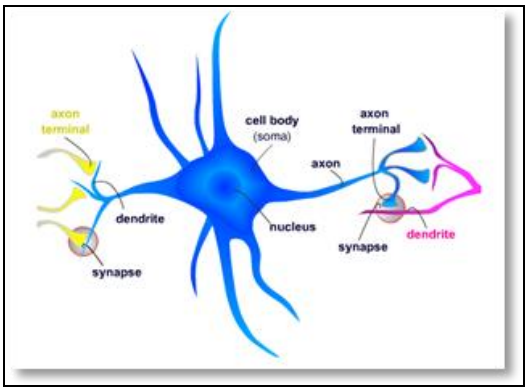


Figure 3-3: The structure of a typical neuron
There are several different types of neurons, but regardless of their function or location all neurons consist of dendrites, a call body, an axon and axonal terminals which through the transmission of neurotransmitters allow for communication between neurons. Image from Stufflebeam (2008).

A message is received in a neuron when chemicals - or neurotransmitters enter the neurons dendrites. Once in the dendrites, the signal flows through a series of changes in the neurotransmitter through to the cell body and down the axon. The neurotransmitters are then released from the axon terminals to the dendrites of the next neuron (Stufflebeam, 2008) and the signal continues.

There are several different types of neurons but the majority of neurons are either projection neurons or interneurons. Projection neurons only communicate with other projection neurons and are far reaching, their axons project to the cortical regions of the brain and to the spinal cord. Projection neurons can be either motor or sensory in nature. As their names suggest, motor neurons convey motor information while sensory neurons convey sensory information throughout the nervous system. Interneurons interconnect with all other neurons and cover only a small distance within the brain (Stufflebeam, 2008).

In Parkinson's disease it is the far reaching projection neurons that are especially vulnerable to Lewy body pathology – particularly those which have disproportionally long

and thin axons in relation to the size of the cell soma. An exception to this is those projection neurons which maintain a well-developed myelin sheath, as this serves as a neuro-protective feature against the formation of Lewy pathologies. Projection neurons with short axons are less affected by Lewy neurites and Lewy bodies (Braak, Bohl, et al., 2006).

Each thalamic nucleus contains both projection and interneurons. Thalamic regions that are targeted by LB pathology may therefore show differential levels of degeneration depending on the quantity of projection neurons in each area. The majority of neurons in the ventral anterior nucleus (Hani, Al-Haidari, & Saboba, 2007), for example, are projection neurons with the remainder of the nucleus consisting of interneurons – suggesting that this region may show higher neuronal loss than some other regions. In contrast, the ventral lateral and ventral posterior regions of the thalamus may not be as affected by LB pathology as there are a smaller number of projection neurons than interneurons in these regions (al-Hussain, 1992). The opposite is true for the remaining thalamic regions (Armstrong, 1990; Steriade, Jones, & McGormick, 1997) however, so in the majority of thalamic nuclei LB pathology may be a strong influence on neuronal death.

3.2.4 The information processing model

A disruption to any part of the communication system that the thalamus is part of will result in functional deficits. This is because the structures of the brain do not contribute to behaviour and cognition independently but instead have several connections, many of them reciprocal between each other and the wider cortex. A breakdown in a substantial number of these connections leads to deficits in cognitive, and other behavioural functions (Rub, Del Tredici, Schultz, et al., 2002). The general association between cortical areas and certain cognitive processes is well established. For example, damage to the frontal cortex has long been associated with attention deficits (Heilman & Valenstein, 1972) and damage to the temporal lobe with memory deficits (Alvarez & Squire, 1994). These broad associations can be further narrowed to the smaller subcortical structures within these areas. Within the temporal lobe, for example, the progressive relationship between degeneration in the subcortical hippocampal structure and the decline in semantic memory in Alzheimer's disease can predict conversion from MCI to AD (Apostolova, et al., 2006).

In regards to the thalamus – the primary connections that each thalamic region has with specific areas within the neocortex is considered to be influential over those processes that are supported by that region. Additionally, the connectivity that a thalamic region has

with other subcortical structures before the fibre connections reach the neocortex is also influential over those processes mediated by that structure. A breakdown in the connectivity between the thalamus and the hippocampus, for example, would impact on memory function – even if the hippocampus itself remained intact.

An influential model which presents the theory of dysfunction as arising from the disruption in connectivity in PD is the information processing model (*Figure 3-4*), proposed by Rub et al., (2002). They theorised that Lewy body pathology in subcortical regions alone cannot cause the myriad of symptoms in PD and instead, it is the disruption in communication between key areas that is thought to be a stronger contributor to PD symptoms.

In the itnralaminar regions of the thalamus for example, the degree of LB burden is heavy. Neuropathology here could cause neuronal death in this region which would, in turn, affect the axonal connectivity the itnralaminar region has with the surrounding hippocampal region and the anterior cingulate and prefrontal cortex. This axonal disruption prevents the normal communication process and is related to the frontal symptoms of PD (Beyer, Janvin, et al., 2007).

The idea of LB pathology affecting neuronal degeneration is also supported by histological studies conducted by Henderson, et al., (2000a). Although this group identified a great degree of neuronal loss in subregions of the thalamus in their PD sample, LB's were only present in a small proportion of the remaining neurons. They suggested that this high degree of cell loss with relatively low level LB's showed that LB's caused neuronal death. LB infiltration in the neuron was thought to lead to the death of that neuron, resulting in the reabsorption of both neuron and LB debris, leaving no neuron and no trace of LB remaining in the thalamus. This outcome suggests that neuronal loss could be a better marker of disease progression than level of LB's as the LB level may change depending on whether neuronal death has occurred.

In regards to the breakdown in connectivity, LB pathology alone may not cause the axonal degeneration of affected neurons, but when the cell body of the neuron dies, so too does the axon arising from that cell body. This has been established to occur in patients with frontal lobe tumours (Hulshoff Pol, et al., 2000). After removal of the tumour, axonal density in the affected area was also severely reduced. As a consequence, the areas the axons projected to also had significant cell loss. Thus, neuronal loss in the thalamus could restrict subcortical-cortical connectivity as axons degenerate and the communication between key areas is reduced.

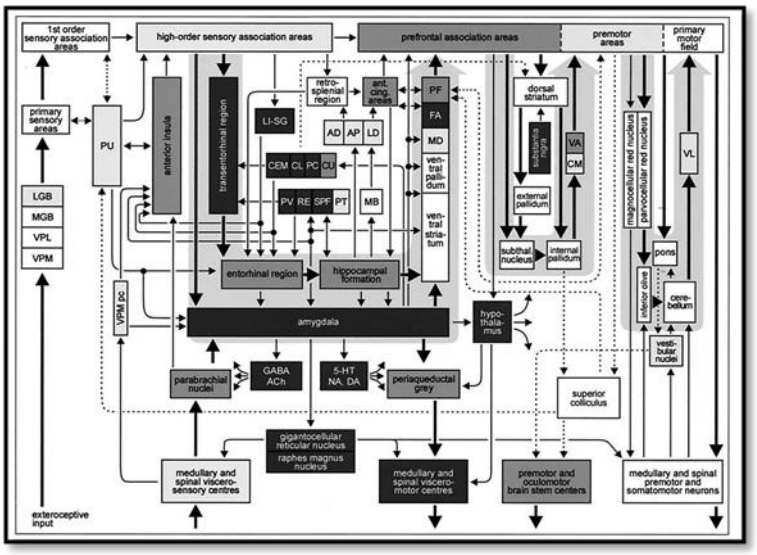


Figure 3-4: The information processing model in Parkinson's disease
Lewy pathology throughout the subcortical and cortical structures in Parkinson's disease causes a system wide disruption in cortical communication. The nuclei of the thalamus are key components of the striatal, cerebellar and limbic loops – contributing to the autonomic, emotional and cognitive symptoms of PD respectively. Image from Rub, Del Tredici, Schultz, et al., (2002). The degree of LB burden is indicated using a progressive shading scale where white is no LB burden and black is heavy burden.

3.3 The influence on cognitive dysfunction

Given the location and connectivity of the thalamus, it is not surprising that damage to this area results in a myriad of symptoms. Primary evidence for the thalamic contribution to cognitive decline comes from individuals who had suffered thalamic infarction after a stroke but who are otherwise neurologically intact. Severe memory loss has been reported as a consequence of thalamic damage in the majority of these individuals, with the anterior and medial portions of the thalamus most involved in this aspect of cognitive dysfunction. The anterior nuclei are the strongest contributors to amnesia and anterograde amnesia is normally only seen in patients where thalamic damage is concentrated to areas of the anterior thalamic nuclei or in the mammillo-thalamic tract (Carrera & Bogousslavsky, 2006). Mammillo-thalamic tract damage is of particular interest because it is this white matter bundle that projects from the anterior thalamus to the cingulate gyrus, hippocampus and the orbitofrontal and prefrontal cortices, mediating both memory and executive function processes (Mori & Hashimoto, 2001).

Memory deficits after thalamic infarct has also been reported in another cohort (van der Werf, et al., 2003) of individuals with thalamic lesions. The exact location of the lesion site was able to be verified using MRI, and in the cases where the lesion site was restricted to a specific sub region of the thalamus - any cognitive or functional changes of the patient could

be attributed to that individual region. Within this cohort, those who had thalamic lesions but no other cortical damage were isolated. It was found that although damage to the mamillo-thalamic tract predicted memory deficits, no single area in the thalamus accounted for memory impairment. If there was damage to several areas within the thalamus such as within the anterior and the medial regions however, this significantly contributed to amnesic symptoms. Conversely, lesions within the posterior and lateral thalamus could be present without any memory deficits arising. Although the executive functioning and attention of the patients was also examined, impairment on these tasks could not be attributed to any single region of the thalamus (van der Werf, et al., 2003). Evidence from this study supports the idea that thalamic regions are functionally specific and although each will primarily be involved in separate areas of function, the combined influence of thalamic nuclei is greater than the sum of its parts due to the extensive connectivity each thalamic region has with other subcortical structures.

An additional study (Carrera & Bogousslavsky, 2006) conducted on patients with post-stroke thalamic infarcts also supports the idea that thalamic damage in isolated regions results in the varied presentation of cognitive deficits between patients. Infarcts in the anterior thalamus, for example are well established to result in amnesic syndrome and patients with MRI proven anterior infarcts in this cohort also suffered from intrusions of unrelated topics, distorted memories, short-term amnesia and executive dysfunction. Anterograde amnesia, with recognition preservation was also a common result of an anterior thalamic infarct in these individuals, with symptoms continuing to persist several years after the stroke. Hemisphere of the affected thalamus is also relevant as visuospatial deficits (Graff-Radford, Eslinger, Damasio, & Yamada, 1984) have been reported in patients with infarcts restricted to the right thalamus and verbal impairment (Clarke, et al., 1994) reported in those with predominately left thalamic damage.

The dorsomedial regions have also been implicated without anterior involvement in memory impairment in other samples. Infarcts that span both the combined dorsomedial and centromedian regions of the thalamus show a relationship with amnesia (Castaigne, et al., 1981) and memory/attention impairment (Gentilini, De Renzi, & Crisi, 1987). Disinhibition and other behavioural changes are also widely reported after infarcts in these areas (Bogousslavsky, et al., 1988). The effect of the dorsomedial and centromedian regions are also able to be further dissociated, as patients with pure centromedian involvement present with discrete amnesia but also severe distractibility (Mennemeier, Fennell, Valenstein, &

Heilman, 1992), suggesting that attention deficits may be underlying the presentation of memory deficits here. Patients with lesions in only the dorsomedial area, on the other hand, present with amnesia less severe than those in the anterior thalamic region but nevertheless also show symptoms of executive dysfunction, anterograde amnesia and – for those patients with left sided lesions, aphasia (Carrera, Michel, & Bogousslavsky, 2004).

The difference in patient presentation of lesions located to the dorsomedial and anterior thalamic regions can be easily attributed to their primary connectivity with separate regions of the cortex. Both involved in the limbic loop (*Figure 3-4*), the mediodorsal thalamus has direct connectivity with prefrontal association areas, mediating attention and executive function processes while the anterior thalamus is primarily connected with the hippocampus and mediates memory processes.

In addition to this, infarcts in the ventral lateral regions of the thalamus have also been reported to affect executive function – a process that spans both attention and working memory. Patients with lesions in these regions showed impairment on tests of verbal and figure fluency and performance of the Stroop attention and processing task (Annoni, et al., 2003). As these cognitive changes have also been reported after cerebellum lesions (Schmahmann & Sherman, 1998) these deficits are thought to mainly arise due to impairment in the connectivity of the ventral lateral regions with the cerebellum.

3.4 Thalamic changes in other neurodegenerative disorders

Changes in the macro structure of the thalamus have been identified in other neurodegenerative disorders such as multiple sclerosis (MS: Houtchens, et al., 2007), Huntington's disease (Kassubek, et al., 2005), and Alzheimer's disease (de Jong, et al., 2008), with microstructural changes also evident in AD (Cherubini, et al., 2010). Despite the variation in the clinical presentation of these disorders, they all show some degree of cognitive dysfunction - although the type and degree of this varies. In Huntington's disease for example, the main area of cognitive impairment is in the domain of executive function while in multiple sclerosis cognitive deficits are mainly of attention and episodic memory (Deloire, et al., 2005). In AD-MCI cognitive impairment manifests as a decline in memory function with all other areas of cognition preserved. Once these patients progress to dementia, all areas of cognition become progressively worsened, especially in the language and visuospatial domains (Lambon Ralph, Patterson, Graham, Dawson, & Hodges, 2003). The thalamus, having been implicated in all the above areas of cognition (van der Werf, et al.,

2003) is the underlying common factor in all these disorders, suggesting that the type and severity of cognitive function in neurodegenerative disorders may reflect the extent, location and degree of cell loss in the thalamus.

3.4.1 Volume

Cell loss can be measured at autopsy (Halliday, 2009) with an *in vivo* representation of cell loss also able to be visualised using MR imaging. Atrophy of the thalamus for example is assumed to represent the destruction of neurons and axons (Houtchens, et al., 2007) and can be measured using either manual (Houtchens, et al., 2007), or automatic (de Jong, et al., 2008) methods from structural (T1) MRI images in either native subject (Houtchens, et al., 2007) or standardised (Kassubek, et al., 2005) space.

3.4.1.1 Huntington's disease

In Huntington's disease, standardised voxel based morphometry methods have typically been applied. This involves the image from each subject being mapped to the same stereological space with the average thalamic volume of the disease group then able to be compared to the average of the control group. Results show that, on average, the thalamus has markedly decreased grey matter density compared to that of the control subjects in this cohort (Kassubek, et al., 2005). Loss was concentrated in large areas of the dorsomedial, ventrolateral and centromedian regions of the bilateral thalamus, occurred independently of normal aging and had a significant association with performance on tasks of executive function.

3.4.1.2 Multiple sclerosis

In the multiple sclerosis sample (Houtchens, et al., 2007) the thalamus was measured using manual tracing methods in subject native space. A 16.8% reduction in thalamic volume was reported in the MS group relative to control subjects after normalisation for intracranial volume (ICV). Thalamic volume reduction was associated with impairment on cognitive tests of processing speed/working memory and visuospatial memory in the MS group. Thalamic volume in this sample predicted cognitive function better than other MRI measures of third ventricle width, lesion volume and whole brain atrophy. In another MS sample slowed cognitive processing has also been independently associated with thalamic atrophy (Batista, et al., 2012). After controlling for the influence of neocortical and basal ganglia volume,

thalamic volume was found to be significantly independently associated with multiple measures of information processing and speed performance in the patient sample.

3.4.1.3 Temporal lobe epilepsy

Thalamic degeneration and the association with cognitive dysfunction has also been implicated in those with temporal lobe epilepsy (TLE), a disorder which mainly causes memory deficits due to the reoccurrence of seizures that induce alterations in neural circuits (Pitkanen & Sutula, 2002). Using manual tracing methods to delineate the thalamus and hippocampus of patients, Seidenberg et al., (2008) showed reduced thalamic volume in the patient group relative to healthy controls. Furthermore, this study indicated differential involvement of the thalamus depending on the hemisphere it was located in. The left thalamus was the best predictor variable of verbal IQ in patients with right TLE and the right thalamus the best predictor of verbal IQ and performance IQ in those with left side TLE. In a different sample of TLE patients (Stewart, et al., 2009) thalamic volume was compared with a healthy control group, and although volume loss was only evident at trend level, there was a significant association between volume and a test of memory recall. The volume of the thalamus continued to be an independent predictor of this score even after controlling for hippocampal volume.

3.4.1.4 Schizophrenia

Several studies have examined thalamic volume in Schizophrenia due to the reciprocal connectivity the thalamus shows with the cortical regions that are implicated in the disorder. The prefrontal cortex, for example has a strong association with some of the negative symptoms of Schizophrenia such as deficits in emotional responses and other thought processes which present as apathy, blunted affect or a lack of motivation (Martino, Bucay, Butman, & Allegri, 2007) and is also reciprocally connected with the anterior and medial regions of the thalamus (Jones, 2007b 2007a). Nearly all Schizophrenia samples point to a significant bilateral (Brickman, et al., 2004; Gilbert, et al., 2001) or left (Qiu, Zhong, et al., 2009) thalamic reduction in patients relative to control subjects which survives correction for ICV in all but one (Csernansky, et al., 2004) sample. Only some studies have gone further and examined the relationship between reduced thalamic volume and impaired cognition in schizophrenia, finding a positive association with executive function measures (Batista, et al., 2012) and executive function and language (Coscia, et al., 2009). In one of these samples,

(Qiu, Zhong, et al., 2009), although the shape of the thalamus was significantly correlated with measures of spatial memory and executive function, the volume of the thalamus did not have a relationship with any measure – suggesting perhaps that the memory and executive function deficits in this sample were influenced by regional thalamic changes rather than gross thalamic atrophy.

3.4.1.5 Alzheimer's disease

In contrast, when thalamic volume has been examined in Alzheimer's disease samples there is a consistent deficit in volume loss which has a strong relationship with cognition. Thalamic involvement in AD is of particular interest as, traditionally it is the hippocampus that is considered to be the main biomarker of cognitive impairment (Apostolova, et al., 2006; Jack, et al., 1999) as AD mainly presents as memory dysfunction (Bronnick, Emre, Lane, Tekin, & Aarsland, 2007). Given that AD is not a disorder of 'pure' memory deficit however it is not surprising there is also some degree of thalamic involvement in cognitive symptoms. Thalamic volume has been reported to be significantly reduced in a sample (Stout, et al., 1999) of AD patients and has a strong relationship with cognition. Thalamic volume loss in this sample was measured after segmentation of images into grey and white matter and the subcortical structures were manually delineated from the grey matter maps. Thalamic atrophy had an independent association with the learning, delayed recall and recognition trials of the California Verbal Learning Test. Learning of the CVLT word list was found to be more impaired when damage to the thalamus or surrounding medial temporal cortex was more severe than damage in the neocortex, indicating that thalamic damage in this case is a stronger contributor to learning and memory than damage to the grey matter of the wider cortex and perhaps occurs first.

Some AD samples show significant thalamic volume loss, but no association with cognition. In a different sample of AD patients (Callen, Black, Gao, Caldwell, & Szalai, 2001), the thalamus showed a significant volume loss compared to healthy control subjects. This study was somewhat unique in that it measured all structures of the limbic system (basal forebrain, cingulate, hippocampus, mammillary bodies, orbitofrontal cortex, parahippocampal cortex, septal area and thalamus) rather than just concentrating on the hippocampus. Although all regions except the anterior cingulate showed a significant degree of volume loss in AD, the degree to which a particular structure was involved varied. The hippocampus for example, showed the greatest volume loss of 28.30% and was also the only subcortical

structure which, alone, could discriminate between the patient and control group with a significant degree (85%) of accuracy. The thalamus was not an independent predictor of cognition and did not appear to contribute to cognitive dysfunction. Although there was a significant volume reduction of 12.7% this measure could not accurately discriminate between the patient and control groups, even when examined in the same model as other subcortical structures.

Unfortunately only the Mini Mental Status Exam (MMSE) and the Dementia Rating Scale were applied as cognitive measures in this sample so it is difficult to determine exactly the neuropsychological profile of this cohort. Given that a progressive memory deficit is required for the diagnosis of AD and is generally the first symptom a patient will report (Aarsland, Cummings, & Larsen, 2001) it is likely that only the hippocampus was mainly involved at this stage of the disorder as patients may not have yet been exhibiting large deficits in any wider areas of cognition.

In support of this idea, several studies have shown that thalamic volume appears to only be reduced when memory deficits are severe, which could explain the inconsistency between results in the Callen, et al., (2001) and Stout, et al., (1999) samples. In a study of those that self reported memory deficits (de Jong, et al., 2008), for example, thalamic volume was not found to be significantly reduced despite the fact patients felt they had memory impairment. Conversely, in the same study, the thalamus showed a significant volume reduction in those with diagnosed AD. The self report group was classed as 'memory complainers' rather than healthy controls in this case as they presented with self reports rather than objective measures of memory impairment. Deficits were at a sub-clinical level however as no decline was evident when the group was tested using objective memory measures. Deficit was examined using both the MMSE and the Cambridge Cognitive Examination-Revised (CAMCOG-R: Roth, Huppert, Mountjoy, & Tym, 1999). The insensitivity of the MMSE to mild forms of cognitive impairment in PD (Zadikoff, et al., 2007) has already been discussed and may have also missed MCI in this sample, classifying patients as exhibiting normal cognition when, in fact they were not.

There are also some reports that suggest the CAMCOG-R faces this problem, showing low accuracy in discriminating between MCI and control subjects, although it can discriminate between MCI and dementia with more success (Nunes, et al., 2008). Another difficulty in interpreting this study comes from the lack of healthy control group. The memory complainers group may, in fact, have been exhibiting volume loss that was

intermediate to that of normal controls and the AD group. Regardless of whether the subtleties of mild cognitive impairment was missed in the 'memory complainers' or if they were in fact of a normal cognitive status, these results merely confirm that the thalamus shows gross atrophy if cognitive impairment is severe. If these participants were in fact in the early stages of AD it only adds to the conclusion that the thalamus is significantly degenerated in dementia, over and above what may be expected as a consequence of normal aging. Even after adjustment for age, gender and ICV in this sample, bilateral thalamus was reduced in the AD group relative to the comparison group. Furthermore, in the AD group, volume reduction in the left was correlated with poor executive functioning scores while volume reduction in both left and right thalamus was correlated with poor scores of global cognition.

3.4.1.6 In Mild Cognitive Impairment

Thalamic volume has been examined in other cohorts exhibiting MCI but inconsistent results are reported, further suggesting that thalamic volume loss is only evident when cognitive dysfunction is severe, or that the volume varies according to the criteria that is used to diagnose MCI. In one sample (Karas, et al., 2004), for example the idea that the thalamus shows significant levels of degeneration in the early stages of AD was confirmed. From a whole brain voxel based analysis, the thalamus was identified as the region most affected in MCI compared to healthy control subjects. MCI was diagnosed according to Petersen criteria (Petersen, et al., 1999) which states MCI is present if there is an impairment of memory (1.5 SD below age adjusted means of normative data adjusted for age) but general cognitive function is normal. Between MCI and AD, although the greater temporal lobe showed significant degeneration in this study there was no further thalamic degeneration, suggesting that the greatest disruption of the thalamus occurs early in the AD process. Further degeneration may progress in an outward pattern from here and manifest as further cognitive impairment as additional regions become involved (Braak & Braak, 1996).

In a similar MCI cohort (Pennanen, et al., 2005) volume loss was identified using voxel based analysis in regions of the thalamus, along with the hippocampus, amygdala, anterior cingulate and parietal and temporal lobes compared to healthy control subjects. The authors of this study did not examine the relationship between volume of the ROI's and cognition but, as MCI was diagnosed according to criteria proposed by the Mayo Clinic Alzheimer's Disease Research Centre (Petersen, et al., 1995) as impairment in one memory or

any one other cognitive domain it is safe to assume that cognitive dysfunction was influenced by volumetric degeneration in subcortical structures and was not an artefact of normal aging.

In line with this, a recent AD sample (Roh, et al., 2011) that did not differentiate between MCI and AD but included patients across the whole spectrum of cognitive dysfunction (CDR range 0.5-2) found thalamic volume was reduced as a function of disease severity but that this only reached statistically significant levels when severity was 1 on the CDR scale. Thalamic volume did have a strong positive relationship with all areas of cognition (attention, language, visuospatial function, memory, frontal executive function) both independently and as an aggregate measure in this sample. A further AD sample also showed that thalamic degeneration was only identifiable once cognitive impairment was extreme, with significant atrophy evident in the AD group relative to controls but not in the MCI group (Cherubini, et al., 2010). This last sample also diagnosed MCI according to the criteria of (Petersen, et al., 1999) but thalamic volume was measured using an automated procedure individually for each subject – perhaps suggesting that the previous results are spurious and mainly the result of the standardisation procedure.

Arguably the most informative methodology for thalamic involvement in the progression of MCI to AD is that that is gained from longitudinal studies. Studies of this kind first examine the thalamus in a MCI cohort and then that same cohort is followed up some time later and differences between those who have developed AD are compared to those that have not. One such study (Chetelat, et al., 2005) uses voxel based methods and provides strong evidence for the involvement of the thalamus in those with MCI who later convert to Alzheimer's disease. Eighteen patients with amnesic MCI were followed for 18 months to determine if any neurological differences between those who developed Alzheimer's disease in this time period existed compared to those who did not. Although the MCI group were not initially compared to a control group, at follow up, six of the eighteen patients who had initially been diagnosed with MCI had converted to AD. Over time, the areas of greatest grey matter loss in the combined converters and non converters group included prominent areas within the temporal, frontal and parietal lobes. The only subcortical structure implicated was the left thalamus. In converters compared to non converters, there was accelerated grey matter loss concentrated in the inferior and middle temporal neocortex, indicating that although the atrophy process is similar in both groups it occurs faster in the converters.

A similar longitudinal study (Fischl, et al., 2002) that did include a control group supports these results. Delineation of the thalamic structure was achieved with an automated

segmentation procedure which allowed for simultaneous examination of ventricles and the hippocampus alongside the thalamus. Patients were initially categorised as those with AD and those with questionable AD at baseline based on the clinical dementia rating scale. Those with questionable AD were further subdivided into either converters or non-converters at three year follow up examination. Converters were those that progressed from questionable AD at baseline to the National Institute of Neurological and Communicative Disorders and Stroke/Alzheimer's Disease and Related Disorders Association criteria for probable AD. Thalamic volume (defined at initial examination) was then compared cross-sectionally across the converters, probable AD and control subjects. The thalamic volume of the converters and the AD patients was significantly reduced compared to control subjects. Subcortical volumes were not re-examined at follow up. The fact that the thalamus was showing significant volume reduction before AD was diagnosed indicates that thalamic disruptions occur prior to the appearance of clinical symptoms. The third and lateral ventricles also showed significant increases in the converters and the AD patients relative to the control subjects, indicating that thalamic degeneration is accompanied by ventricular enlargement in this case.

3.4.2 Cellular microstructure

Although, it is perhaps now evident that the thalamus shows some degree of degeneration prior to the onset of dementia, inconsistent methodology and criteria for MCI renders these results questionable. MR technology has significantly advanced in recent years however and now, alongside gross structural abnormalities the subtle changes at the cellular level are also able to be examined. As subtle changes in thalamic tissue composition may not necessarily result in gross structural volume loss examination of the thalamus at the cyto-archetecture level is invaluable.

The MR technique of diffusion tensor imaging (DTI) is able to provide evidence of tissue disruption where conventional measures of atrophy may fail to detect size differences. This has been demonstrated in Schizophrenia (Agarwal, et al., 2008; Rose, Chalk, et al., 2006) and in AD-MCI (Cherubini, et al., 2010; Rose, McMahon, et al., 2006).

3.4.2.1 Schizophrenia

In one of the schizophrenia samples (Agarwal, et al., 2008) there were subtle cellular abnormalities in the thalamus even though overall thalamic volume was maintained. This cohort consisted of 71 patients with schizophrenia and 75 control subjects. The diffusion

measure was the apparent diffusion coefficient (ADC) which provides a measure of the average rate and degree of diffusion through specific regions of the brain and takes into account barriers to the normal movement of water such as cellular structures. The ADC can also be referred to as mean diffusivity, the two terms are interchangeable (ADC = MD). In the schizophrenia patients, the rate of diffusion through the thalamus was significantly higher than it was through the thalamus of the control subjects. The volume of the thalamus on the other hand, only showed a trend for volume loss – and only in the left thalamus. Neither measure showed a relationship with the clinical symptoms of Schizophrenia, although both diffusion and volume was correlated with the age of the participants.

A second schizophrenia sample (Rose, Chalk, et al., 2006) also reported significantly elevated diffusion within the thalamus. In this sample, diffusion increases (MD) were concentrated to anterior and medial thalamic regions. No analyses in relation to the association this may have had with any aspects of function were reported.

Results are still somewhat controversial in schizophrenia, as in patients with first episode schizophrenia, the opposite effect is present (Qiu, Zhong, et al., 2009). Significant volume loss, but no changes in MD has been reported in one patient sample relative to control subjects. This study also examined the fractional anisotropy (FA) measure of diffusion – a measure of the direction of diffusion which infers the integrity of the underlying cell structures. A higher FA value indicates that diffusion is occurring in one main direction and shows that the cell structures are mostly intact. As the water follows the direction of cellular structures, if they were damaged the water would be free to move about in any direction, and give a lower measure of directionality (R. Watts, 2008). There were no differences in FA values in the thalamus of the Schizophrenic group compared to the normal control subjects although the level of FA in this cohort was associated with deficits in spatial working memory. The authors suggest this result, which is in contrast to both previous studies which have examined diffusivity parameters in Schizophrenia is due to the fact their cohort was comprised of first episode patients. These patients had not yet undergone any significant pharmacological alterations that may have occurred due to antipsychotic medications. Caution then needs to be undertaken when interpreting results of cohorts that may be subject to intense intervention.

3.4.2.2 Alzheimer's disease

Results are more consistent in Alzheimer's disease. In AD the integrity of the thalamus is shown to be significantly disrupted at the first signs of cognitive dysfunction, before degeneration has reached the cortex. In patients with amnesic MCI for example, several subcortical structures, including the right thalamus showed significantly reduced FA while most of wider cortices showed no changes (Rose, McMahon, et al., 2006). In the sample of MCI patients already mentioned in the volumetric section above (Cherubini, et al., 2010), although significant levels of thalamic atrophy were not detected until AD, prior to this, the thalamus of the MCI group showed significant microstructural degeneration. There was also a significant increase in MD in AD relative to the MCI group, suggesting microstructural changes of this nature could be followed throughout the course of the disease as a neurocorrelate of cognitive dysfunction.

There is one study (Kantarci, et al., 2001) that has reported contrasting results to this in pre AD MCI however. This study did not use standard criteria to quantify MCI so patients with MCI were diagnosed as having subjective MCI if they 1) had a memory complaint (not measured); 2) exhibited global cognition within the normal range, 3) had normal activities of daily living and were not demented. In this cohort of MCI patients compared to healthy control subjects, increased diffusivity was only evident in the hippocampus, with thalamic diffusivity increases evident at trend level only. Once AD had developed, however, increased diffusivity was evident in several further cortical regions (parietal, occipital, temporal cortices and anterior and posterior cingulate) but the level of cellular disruption remained the same in both the thalamus and in the frontal lobes. Given that AD can be considered to be more of a temporal rather than frontal disorder (Apostolova, et al., 2006), this perhaps just indicates that thalamus contributes to some of the deficits in AD but cannot be considered to be an independent factor in the progression of the disorder. Had more detailed neuropsychological information for any of the above samples been available, maybe some relationship between those samples who do exhibit thalamic disruption and those who do not and their cognitive profiles could have been established.

3.5 Summary

As the thalamus is centrally located and connected to most cortical regions this subcortical structure plays some role in most cognitive functions (Taber, et al., 2004). The neurons of the thalamus, especially the projection neurons are targeted by LB pathology in Parkinson's

disease which causes significant levels of cell loss in some thalamic regions (Halliday, 2009; Henderson, et al., 2000a, 2000b). Neuronal loss in the thalamus not only causes significant thalamic disruption but has a follow on effect where multiple cortical and subcortical processes are also affected as the connectivity between the thalamus and the cortex breaks down (Hu, et al., 2001). In other neurodegenerative disorders where similar cognitive deficits are present the thalamus shows a significant degree of volume disruption, associated with cognitive dysfunction (de Jong, et al., 2008; Hazlett, et al., 1999; Houtchens, et al., 2007). Prior to volume loss, the cellular integrity of the thalamus is also disrupted and has a relationship with even subtle levels of cognitive dysfunction in the early stages of some disorders (Rose, McMahon, et al., 2006).

Chapter 4. THE THALAMIC NUCLEI

4.1 Objectives

This review will first examine the anatomy and function of thalamic nuclei. Evidence of changes in pathology, structure and cellular integrity in both thalamic nuclei and their associated projection sites obtained from post-mortem and neuroimaging studies will be discussed. The relationship between thalamic nuclei and behaviour will be explored using evidence from multiple neurodegenerative and neuropsychiatric diseases as well as from case studies where localised brain injury or lesion has occurred. Where possible, studies for review have been restricted to those with structural, and diffusion methodology only. As such a small amount of these have been conducted in cohort of PD participants however, any studies that have addressed changes in thalamic nuclei in PD have been included. Information presented here will aid understanding of *Chapter 6* which address the gross anatomical, cellular and integrity changes of thalamic nuclei and *Chapter 9* which addresses the changes in the integrity of axonal connectivity between thalamic nuclei and projection sites in the cortex. In conjunction, the two studies provide a unique opportunity to investigate the relationship between the thalamus, it's axonal connectivity and the predictive validity this provides for cognition and motor dysfunction in PD.

4.2 Thalamic organisation

The thalamus is organised according to the cyto-architecture of neuronal cell bodies and axonal cortical connectivity and reflects the organisation of the cerebral cortex (*Figure 4-1*). The substructures that comprise the thalamus preferentially connect to one area of the cortex and each primarily serve a specific area of function (Sherman & Guillery, 2004). Nearly all incoming information is routed through one or more groups of the thalamic nuclei before terminating in cortical areas, a highly regimented and organised form of communication which relies on the integrity of not only the thalamic nucleus involved, but also on the integrity of the axons that connect it with other thalamic, subcortical and cortical regions (Jones, 2007a). The majority of the thalamic nuclei are specific, preferentially projecting to independent and separate regions of the cortex which in turn control motor, limbic, association and sensory functions. The motor regions of the thalamus have afferent and efferent connectivity with the primary, supplementary, premotor and cingulate motor areas of the cortex, influencing all the different aspects of motor control (Picard & Strick, 2001). The

limbic regions of the thalamus are reciprocally connected with the sensory association and prefrontal regions of the cortex, primarily influencing memory, specifically episodic memory and the ability to learn (Braak & Braak, 2000). The prefrontal association areas are involved in attention and executive function (Lewis, Dove, Robbins, Barker, & Owen, 2003). The medial and posterior regions of the thalamus which encompass the association nuclei have primary connectivity with the auditory association area in the temporal lobes, the somatosensory association area in the parietal lobes and the visual association area in the occipital lobes (Hendry & Barbas, 2003) and mediate the interpretation of sound, touch and visual stimuli (Nieuwenhuys, Voogd, & van Huijzen, 2008b). In the centre of the thalamus, lies the intralaminar complex which encompasses the centromedian and parafascicular nuclei. These nuclei are non-specific, axons from this area project around several other nuclei before continuing to multiple areas of the wider cortex and influence multiple processes and behaviours.

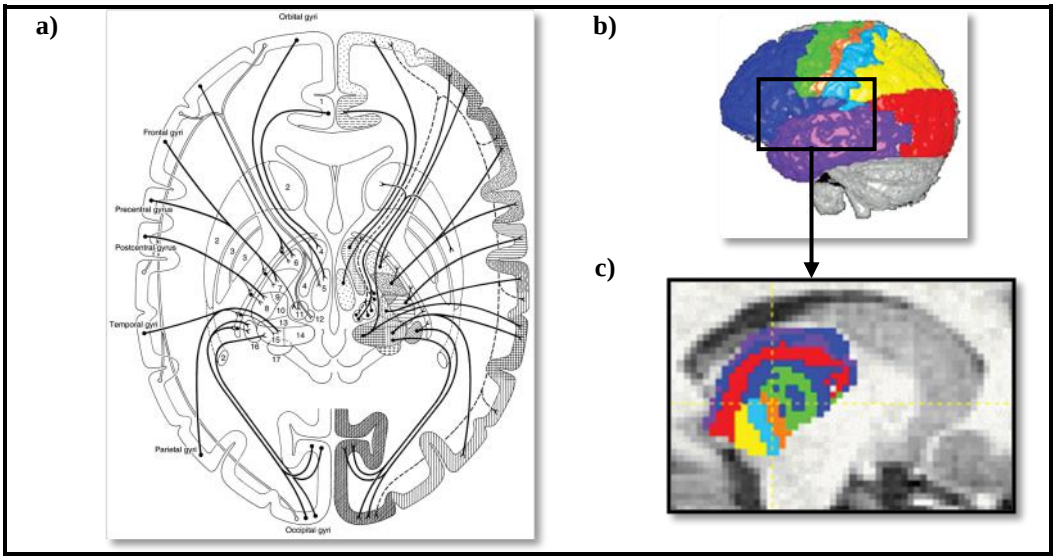


Figure 4-1: The organisation of thalamic components
a) Primary axonal connectivity between thalamic nuclei and the cerebral cortex, image from Nieuwenhuys, Voogd, & van Huijzen, (2008a). **b)** Cortical regions associated with thalamic regions **c)**, image from Behrens, et al., (2003). The thalamus is comprised of several sub regions. The organisation of thalamic sub regions reflects the organisation of the cerebral cortex with each region preferentially projecting to specific regions and influencing specific aspects of behaviour.

4.3 Motor Relay Nuclei

The motor nuclei are perhaps the most widely studied of the thalamic nuclei in Parkinson's disease due to their reciprocal connections with the motor and pre-motor cortex (Nieuwenhuys, et al., 2008b), key areas of functional and structural alterations in PD which result in significant and deliberating motor symptoms. Further subdivided by functionality,

the motor nuclei consist of the ventral anterior (VA) and ventral lateral (VL) nucleus. The VA is principally involved in the execution of movement and the regulation of self-generated voluntary movement while the VL nucleus influences involuntary or automatic movement.

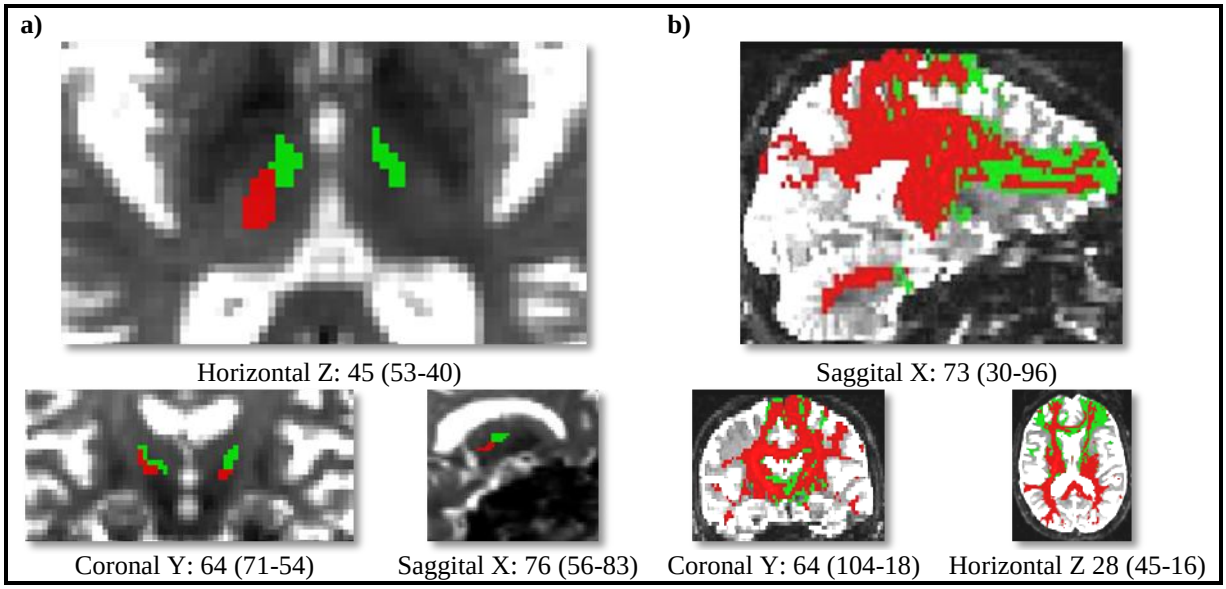


Figure 4-2: Motor thalamic nuclei
Anatomical location (**a**) and connectivity (**b**) of the VA (green) and VL (red) nuclei. In each case the section giving the best view of the regions and their connectivity is presented on the top with views in corresponding slices given underneath. X, Y and Z gives co-ordinates in standard (MNI) space and parenthesis the co-ordinates of the first and final slices where the thalamus is visible. MRIcron images in a) are resliced when opened and are thus displayed using neurological convention, the left side of the image corresponds to the left side of the brain. Images in b) are displayed using radiological convention, the left side of the image corresponds to the right side of the brain.

4.3.1 Ventral Anterior Nucleus (VA)

4.3.1.1 Anatomy, location and connectivity

The VA nucleus is the smallest of the motor nuclei and located in the most anterior position of the ventral thalamus (*Figure 4-2a*). Afferent and efferent connectivity between the VA and cortex occurs by way of the striatal loop (*Figure 4-3*). Originating in the prefrontal cortex, the straital loop passes through the dorsal striatum, an area heavily influenced by the substantia nigra, continues through the pallidum, subthalamic nucleus and VA before terminating in the premotor cortex (Braak, et al., 2000), the area which assums primary responsibility for the generation of movement.

4.3.1.2 Function

The normal function of the straital loop is to initiate self-generated movement through communication with the pre-motor area of the cerebral cortex (Jenkins, Jahanshahi, Jueptner,

Passingham, & Brooks, 2000). Self-generated movement is the preparation for and ability to produce intentional behaviour. This form of movement is internally cued, the individual must decide to perform an action such as reaching for an object or raising an arm, engage in the necessary preparatory procedures for action initiation and then execute it. In contrast, externally cued movement is elicited automatically in response to a stimulus, negating the decision making process (Siegert, Harper, Cameron, & Abernethy, 2002).

While the striatal loop will mediate all forms of self-generated movement the VA nucleus itself appears to be primarily involved in complex movements which have a cognitive aspect, rather than simple repetitive movements. fMRI shows that when healthy control subjects are required to correctly execute a series of finger tapping tasks which were either of a complex nature, requiring several movements which had to be remembered or of a simple nature, requiring only single movements, the VA showed more activation in the complex sequence compared to the simple one (Lehericy, et al., 2006). Although both the VA and the VL project to the motor cortices, the VA directly targets the rostal areas while the VL terminates more caudally. It is this connectivity with the rostal area that appears to be responsible for the mediation for motor tasks with cognitive requirements, as during a complex task the rostal area of the motor cortex shows significantly greater activation than any other region (Haber & Mcfarland, 2001).

4.3.1.3 Motor deficits in PD associated with nuclei dysfunction

In Parkinson's disease there is a significant deficit in self-initiated movement compared to externally cued movement. If a fire alarm was to sound for example, a patient, even if suffering from significant movement dysfunction would get up and walk away much easier than if the movement was internally decided (Siegert, et al., 2002). This deficiency in self-generated movement presents as bradykinesia, one of the cardinal symptoms of PD. Clinically, patients with bradykinesia will experience fatigue, difficulty in initiating movement, completing actions, shifting between motor tasks and will also show an overall reduction in their range of movement (Fahn, 2003).

4.3.1.4 Associated areas of PD pathology and degeneration

Deficit in self-generated movement is associated with reduced activation in the pre-motor regions of the cerebral cortex in PD patients. Compared to healthy controls, reduced brain activity in this area in PD is event, most likely due to a disruption in communication within

the straital loop, as no other regions showed activation during this task. In contrast, during an externally cued task the brain regions of patients were no different to those of control subjects (Jahanshahi, et al., 1995). Intact functioning of the components within the striatal loop and the pre-motor regions of the cortex, therefore, are primarily responsible for self-generated movement and have little influence on externally cued movement.

The VA nucleus only suffers from slight Lewy pathology (Henderson, et al., 2000b) all other subcortical components of the striatal loop - except the substantia nigra are relatively spared until infiltratio of the motor cortex in the final stages of PD. Although the substantia nigra is heavily infiltrated prior to the development of motor symptoms (Braak & Braak, 2000) there is no reduction in the density or distribution of neurons in either the striatum or the globus pallidus (internal and external components) of the straital loop of PD or PD-D patients (Goto, Hirano, & Matsumoto, 1990).

The structures within the straital loop appear to be relatively intact, disrupted connectivity between these regions and the cortex is therefore the most likely the reason for impairment in self-generated movement in PD. Evidence from a fMRI study conducted on PD patients in a resting state supports the theory for disrupted connectivity. Wu et al., (2009) examined a sample of PD patients and healthy control subjects at rest to determine the degree of disruption in the motor circuit. Hypothesising that motor deficits arise from interactions in the motor network rather than from disruption in a singular area they chose to examine subjects at rest instead of during a specific motor task. This allowed for greater interpretation of the whole motor network as, by it's nature, examination of fMRI responses during performance of a single motor task limits interpretation to only those regions involved in that specific task. Compared to control subjects, functional connectivity within the entire motor network was significantly reduced in patients, affecting connections to the supplementary motor area, prefrontal cortex and left putamen. Degeneration in dopaminergic neurons appeared to be the primary cause of disruption, when L-dopa was administered, the pattern of function in patients returned to that of the normal control subjects. The degree of disruption related to the severity of motor impairment, implicating the straital loop rather than only the VA in the observable motor deficits in PD.

Further evidence for both disrupted structure of the VA and disrupted connectivity confirms the above results. During the performance of pre-learned but self generated motor tasks in an fMRI study, the VA nucleus along with premotor, parietal and motor cortices all showed increased activation in control participants. In PD patients, although similar areas

were activated during the same task there was a significant decrease in activation of the striatum and ventral lateral thalamic nuclei. Decreased activation corresponded to the level of motor impairment as measured by the UPDRS (Mallol, et al., 2007).

4.3.2 Ventral Lateral Nucleus (VL)

4.3.2.1 Anatomy, location and connectivity

The ventral lateral nucleus is the largest of the ventral nuclei and is located directly posterior to the VA (*Figure 4-2*) and anterior to the ventral posterior nucleus (VP). The VL is a primary component of the cerebellar loop (*Figure 4-3*), connecting the pre-motor field to the primary motor field via several regions including the magnocellular nucleus, the pontine grey nucleus and cerebellum (Braak, et al., 2000).

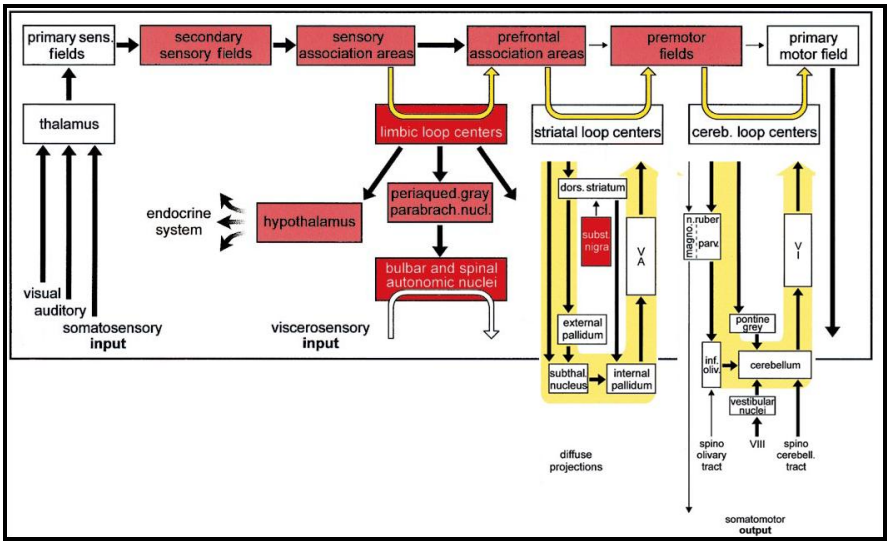


Figure 4-3: The motor nuclei in the striatal and cerebella loops
Diagram modified from Braak & Braak, (2000), the ventral anterior nucleus is a primary component of the straital loop – influencing the initiation of movement while the ventral lateral nucleus is involved in the cerebellar loop, influencing voluntary movement.

4.3.2.2 Function

Normally, the function of the cerebellar loop, especially the ventral lateral component is to modulate the activity of the motor cortex in order to ensure the smooth generation of movement. The intact functioning of the motor cortex enables the appropriate movement to be performed as a response to outside stimuli and is also moderated by mechanisms of bio-feedback, with movement ceasing when the desired outcome is achieved (Blandini, Nappi, Tassorelli, & Martignoni, 2000).

4.3.2.3 Motor deficits in PD associated with nuclei dysfunction

Tremor, a symptom seen in the majority of PD patients is the result of disruption within the cerebellar circuitry. Disruption in any of these components results in less inhibitory signals reaching the cortex and the overproduction of movement. Tremor is the involuntary movement of an affected body part when it is at rest and is commonly the first symptom a patient will present with, although it is not always necessary for diagnosis. Rest tremor is intermittent in the earlier stages of PD, initially only appearing in stressful situations. With the progressive increase of nigrostriatal dopamine loss however, symptoms worsen steadily over time and eventually tremor will occur frequently, continuing to worsen with stress or excitement (Fahn, 2003).

4.3.2.4 Associated areas of PD pathology and degeneration

In this case, it appears the reduction in function of the VL nucleus itself is the primary cause of disruption in the cerebellar circuit. Although Lewy pathology is largely absent from any of the structures within the cerebellar loop (Braak & Braak, 2000), including the VL (Halliday, 2009), the results of lesion studies still suggest that components of the VL are the strongest contributor to involuntary movement in PD. The VL region was the original target for deep brain stimulation in the treatment of tremor, for example. The ventral intermediate nucleus, a region within the VL is the lesion target as damage to this region results in reduction of the frequency and amplitude of tremor in PD. This occurs through the reduction of excitatory signals and the restoration of normal cerebellar communication (Pollak, et al., 2002; Temel & Visser-Vandewalle, 2006). Lesions of the VL significantly decrease tremor but has no effect on bradykinesia, confirming the functional dissociation between these motor nuclei in regards to PD symptoms. Using MRI Duval et al., (2006) conducted a similar experiment, lesioning the ventral intermediate nucleus in PD but with the added advantage of examination of the lesion site both pre and post operatively, a technique that is not normally available until autopsy. Tremor amplitude was significantly decreased in all cases. The imaging showed that although the lesions were comparatively large in this case, encompassing all regions of the ventral lateral nucleus as well as a small region between the VL and VLp nucleus, the VA nucleus was completely unharmed.

4.3.2.5 Summary of the motor nuclei

The motor nuclei are able to be distinguished by their primary function. The VA is involved in the self-generation of movement – the deficit of which results in bradykinesia. In contrast, the VL influences the level of tremor symptoms in PD. The VA is a component of the straital loop, and although showing limited pathology itself, disruption in the connectivity between the straital loop and the pre-motor cortex appears to influence the PD symptom of bradykinesia. Decreased activation of both motor cortices and the VA nucleus is evident in patients compared to controls during movement tasks. The VL also shows limited pathology and neuronal loss, but as lesioning of the intermediate nucleus - a subcomponent of the VL results in the reduction of tremor symptoms of PD, it is degeneration of this region that is thought to be the primary influence on this form of movement dysfunction.

4.4 Limbic Nuclei

Not widely studied in PD, these nuclei nevertheless have important implications in the cognitive aspects of this disorder due to their connectivity with the frontal and prefrontal cortex (Aggleton & Brown, 1999). The prefrontal and frontal regions mediate executive function and learning and memory in healthy subjects, both areas of cognition that PD patients present with early in the disorder (Janvin, et al., 2003) and the biggest predictor of later dementia (Piccirilli, D'Alessandro, Finali, & Piccinin, 1997). With essentially the same regions of connectivity, the limbic nuclei can be differentiated by their cellular make up more easily than their functionality and are divided into the anterior principal nuclear complex (AP) and the lateral dorsal nucleus (LD), both of which project to the prefrontal cortex, cingulate gyrus and enthorinal cortices and subiculum of the limbic system (Nieuwenhuys, et al., 2008b).

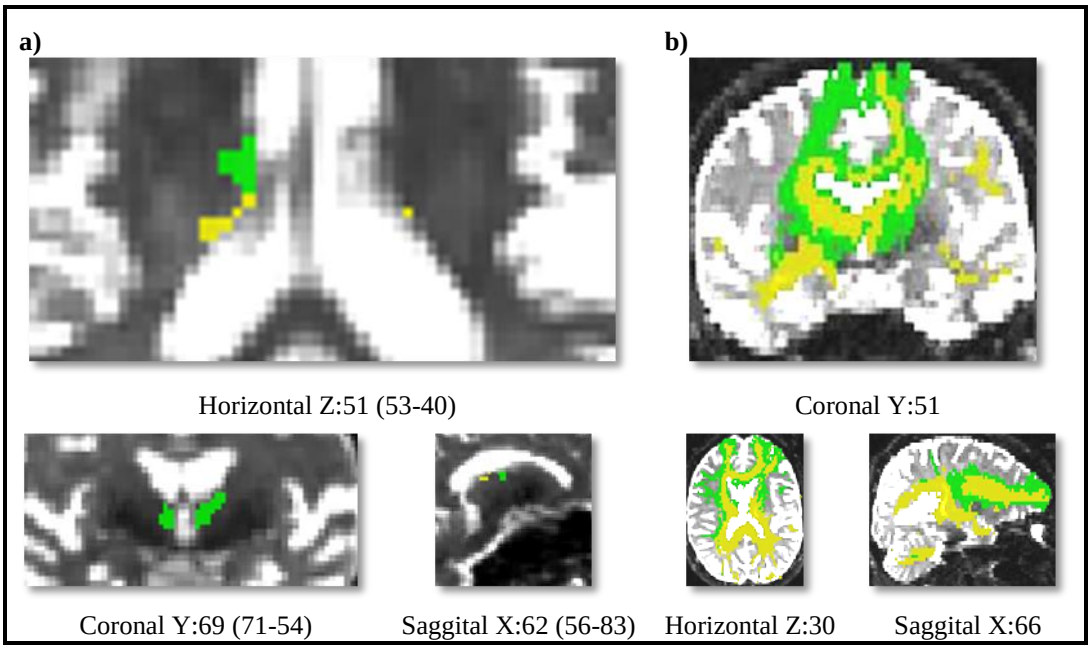


Figure 4-4: Limbic thalamic nuclei
Anatomical location (**a**) and connectivity (**b**) of the AP (green) and LD (yellow) nuclei. In each case the section giving the best view of the regions and their connectivity is presented on the top with views in corresponding slices given underneath. X, Y and Z gives co-ordinates in standard (MNI) brain space and parenthesis the co-ordinates of the first and final slices where the thalamus is visible. MRIcron images in a) are resliced when opened and are thus displayed using neurological convention, the left side of the image corresponds to the left side of the brain. Images in b) are displayed using radiological convention, the left side of the image corresponds to the right side of the brain.

4.4.1 Anterior Nuclear Complex (AP)

4.4.1.1 Anatomy, location and connectivity

The anterior group is made up of the anterior dorsal, anterior medial and ventral anterior nuclei (Aggleton & Brown, 1999). For the purpose of this study the anterior medial and anterior dorsal nuclei will be combined and considered as the 'anterior principal nuclei' as the anterior dorsal nucleus only makes up a small portion of thalamus volume, curving around the anterior ventral nucleus (Zarei, et al., 2009) and is thus difficult to identify on MR images. In addition, there is little observable difference between the cellular makeup of the anterior dorsal nucleus and anterior medial nucleus as both consist of medium sized cells distributed at a moderate density (Jones, 2007b), resulting in similar diffusion parameters and making the segmentation of nuclei difficult. The ventral anterior nuclei will be considered separately. Evidence from animal studies suggests that in order for the full role of the anterior nuclei to be seen, all three of these nuclei must be involved. For example, in rats, lesions restricted to individual nuclei will produce some degree of deficit on a spatial memory task. It is not until these lesions are combined that the full effect of this deficit is seen however (Aggleton, Hunt,

Nagle, & Neave, 1996) so consideration of the anterior medial and anterior dorsal nucleus together will establish the total degree of memory deficit in patients.

The AP nucleus is primarily involved in the limbic loop and connects the sensory association areas with the prefrontal association areas of the frontal cortex. This loop mediates learning ability and memory functions (Braak & Braak, 2000). The mammillothalamic tract, bridging the mammillary bodies and the AP complex is the main input (Van, Saunders, & Aggleton, 2007) while efferent connections from the AP primarily target the anterior cingulate via the retrosplenial region (*Figure 4-5*).

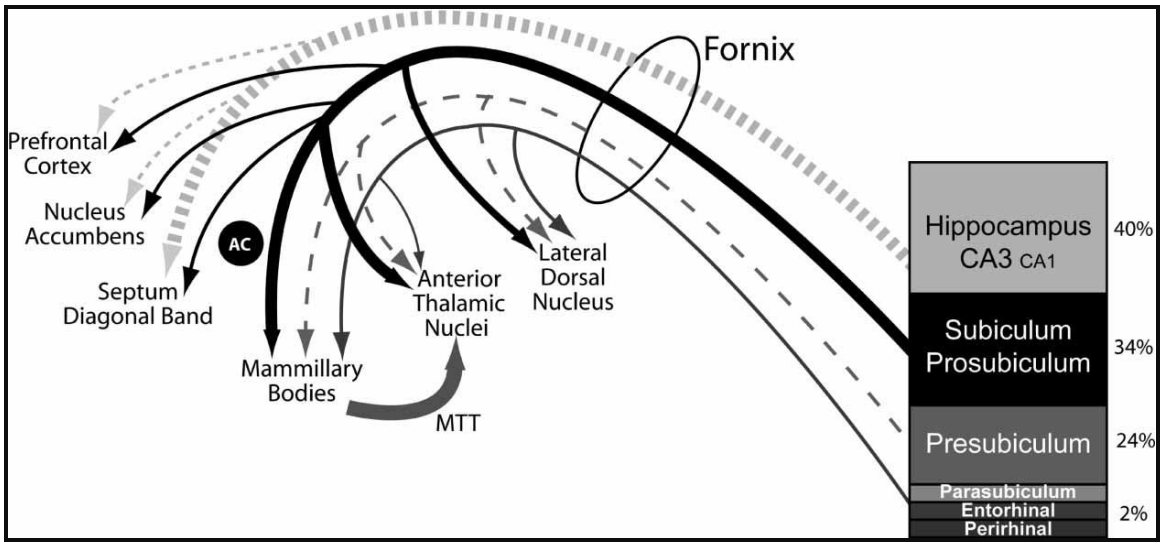


Figure 4-5: Episodic memory
Crucial to episodic memory function, the direct and indirect projections from the hippocampus to the anterior thalamic nuclei via the fornix and mammillothalamic tract respectively comprise the limbic loop. Image from Aggleton & Brown (2008).

4.4.2 Lateral Dorsal Nucleus (LD)

4.4.2.1 Anatomy, location and connectivity

The lateral dorsal nucleus shares similar connectivity with the cortex as the anterior nucleus, the only difference is with the projections received from the mammillary bodies. As with the anterior nucleus, there are reciprocal connections between the LD and the limbic cortex of the cingulate gyrus, retrosplenial area and the subiculum (Nieuwenhuys, et al., 2008b). Due to the anterodorsal location and afferent connectivity from the fornix it is appropriate to consider the LD nucleus, along with the AP as part of the extended hippocampal pathway which mediates the recall memory process (Edelstyn, Hunter, & Ellis, 2006). Our study addresses the AP and LD nuclei separately but as they share similar functionality, deficits in PD associated with these nuclei will be considered together here.

4.4.3 Combined AP and LD Nuclei

4.4.3.1 Function

Intact function of the limbic circuitry primarily serves episodic memory (Aggleton, Desimone, & Mishkin, 1986) with damage to any part of this system resulting in antrograde amnesia (Aggleton & Brown, 1999). There is also evidence of memory and learning associated with the AP being mediated by temporal connectivity as stimulation of the AP results in a reduction of temporal lobe seizures in epileptic patients, indicating connectivity in the temporal lobe alongside the frontal lobes (Fisher, et al., 2010). In line with this, the connective properties of the AP appear to influence the cognitive and behavioural symptoms of schizophrenia, a disorder known for its temporal and frontal disruption (Davidson & Heinrichs, 2003). Histology of the AP in schizophrenic patients reveals there is a significant reduction in neurons compared to control subjects (Byne, et al., 2006; Young, Manaye, Liang, Hicks, & German, 2000). MRI shape analysis has also revealed volume loss in the wider anterior regions of the thalamus (Hazlett, et al., 1999), and the segmentation of nuclei from MRI has identified volume loss in the anterior regions (Gilbert, et al., 2001). Schizophrenic patients in the Hazlett sample had significantly reduced neuropsychiatric scores on executive function and memory measures and in patients it was only the MDn nucleus which correlated with behavioural symptoms, highlighting that the AP was primarily mediating cognitive function in this sample. In addition, the cohort of Gilbert et al., (2001) was drug-naive, first episode patients, indicating that AP volume reduction occurs independently of potentially confounding treatment or duration of illness effects.

The AP has also been implicated in progressive supranuclear palsy (PSP) – a parkinsonian syndrome with motor symptoms similar to Parkinson's disease (Nath & Burn, 2000). Patients showed areas of reduced grey matter within the AP compared to control subjects suggesting that atrophy of this region could also be involved in motor dysfunction. AP degeneration did not exist in isolation in this sample however. The pulvinar and dorsomedial nuclei were also reduced along with other subcortical regions outside the thalamus, suggesting an interaction between areas of degeneration and the clinical presentation of symptoms.

4.4.3.2 Cognitive deficits in PD associated with nuclei dysfunction

Episodic Memory

In Parkinson's disease, episodic memory, specifically the ability to recall word items (Bohlhalter, Abela, Weniger, & Weder, 2009) is one of the earliest cognitive symptoms, appearing well before dementia (Muslimovic, et al., 2005). In the early stages of PD the neuropsychological profile varies between patients so a memory deficit is not always present or necessary for the diagnosis of dementia (Emre, Dubois, et al., 2007; 2007). Patients will always demonstrate some form of fontal syndrome however as, for those who have intact memory, executive function is likely to be impaired (Janvin, et al., 2003). Executive dysfunction is one of the biggest predictors for the development of dementia, indicating that the limbic circuitry is one of the first systems to be affected to a great degree in PD. Patients exhibit deficits on both short term and long term recall of words when asked to recall them freely but markedly less deficit when asked to recall all the items they can remember from a specific subset, e.g.: patients are more likely to recall the presented item of 'apple' when asked to list all pieces of fruit they were presented with rather than when they are asked to list all items regardless of category. Outside of the clinic, recall of day to day events will become impaired but recognition, of family members for example will remain intact (Breen, 1993).

While familiarity of an object appears to be mediated by the MDn, recollection is mediated by the anterior thalamic nucleus and supported by the LD nucleus (Aggleton & Brown, 1999). This influential model of memory dysfunction in relation to the thalamus has recently been investigated using diffusion tensor imaging in patients who have thalamic infarctions (Cipolotti, et al., 2008). Two patients who had localised lesions within the thalamus as a result of stroke were investigated. Both had left MDn nucleus and anterior nucleus and mammillothalamic tract involvement and exhibited deficits on familiarity and recall when examined verbally. Non verbal examination was not in line with the model however, most likely due to cellular disruption in the MTT which was localised to the left hemisphere. In the patient with lesions which encompassed the right MTT, for example, the MDn was spared, dictating that familiarity should have been preserved. The patient, however, exhibited significant deficits when asked if she recognised faces from the Recognition Memory Test. In the patient with damage to the right MDn, the AP and MTT were spared, indicating recollection should have been intact. This patient showed significant deficits when asked to recall both verbal and visual information across all tests including the recognition

memory test and the Rey Complex Figure test. The disrupted the fibre connectivity within the thalamus appears to have a significant influence over cognition and influences, in this case, both recall and recognition.

The dissociation between recall and recognition in PD is most likely influenced by all regions and the connections of the anterior thalamic system. In severe alcoholics with amnesia, damage in the AP alone appears to induce the same cognitive profile that is observed in PD. These patients show amnesic syndrome similar to the memory dysfunction in PD which doesn't appear to be due to the influence of alcoholism. MRI analysis of showed damage was restricted to the AP in amnesic alcoholics compared to alcoholics without amnesia (Harding, Halliday, Caine, & Kril, 2000). The lateral dorsal nucleus also influences symptoms. In a patient with severe bilateral LD lesions which were able to be identified on MRI only recall memory was impaired, leaving recognition intact. The mammillary bodies and fornix were intact and the lesion site completely spared the AP and the main connections to the AP nucleus (only slightly encroaching on left MDn) (Edelstyn, et al., 2006). The LD, is therefore an independent influence on memory, and deficits in this region may be a strong contributing factor to early cognitive impairment in PD.

Executive Function

Executive function deficits are the inability to correctly plan, initiate, monitor behaviour, apply problem solving skills and the flexibility to change behaviour when presented with new information (Leh, Petrides, & Strafella, 2009). Components of the Wechsler Adult Intelligence Scale – IV (Wechsler, 2001) such as letter, action and category fluency or category switching tasks for example are sensitive to executive dysfunction in Parkinson's disease. Clinically, patients with executive dysfunction will have difficulty generating the names of objects within a subset (e.g.: animals) or beginning with a certain letter (e.g.: words beginning with F). Category switching, the ability to rapidly complete a task which intermittently requires the use of different processes or abilities is also an area which shows clinical decline. Measured using trail making tests, where patients are required to link sequential sets of numbers interrupted by an opposing sequential set of letters (e.g. A-1, B-2 etc), patients show significantly more difficulty than healthy control subjects. In daily life, the initiation of behaviour, planning and multi-tasking in patients with PD are the tasks most affected by executive dysfunction. Whereas healthy participants can perform two tasks simultaneously, PD patients tend to focus only on the first task, spending less time and

performing worse on the second in order to adequately achieve the first. This is commonly seen when patients are asked to carry a conversation whilst walking (Koerts, Van Beilen, Tucha, Leenders, & Brouwer).

4.4.3.3 Associated areas of PD pathology and degeneration

There is strong evidence to suggest that disruption in limbic circuitry rather than the AP nucleus itself mediates these frontal cognitive deficits in PD. Although several other structures within the limbic loop succumb to Lewy pathology, the anterior dorsal component of the AP is spared (Braak & Braak, 2000), showing neither volume nor neuronal loss at autopsy (Halliday, 2009). Despite this, there is compelling evidence for AP involvement in the early stages of PD. Episodic memory deficit is a likely result of disruption in the circuitry of the limbic loop. Episodic memory scores in non demented PD patients show a significant positive correlation with blood flow in the bilateral prefrontal cortex (Santens, et al., 2003), the region where the limbic loop terminates. Similarly, executive function deficits have an association with reduced ventrolateral and dorsolateral prefontal activity in PD patients when examined using fRMI during a working memory task. Those without executive function syndrome demonstrated activation similar to that of control subjects. As patients were grouped according to those with executive function deficits and those without but matched in all other neuropsychological tests this suggests preferential involvement of the AP nucleus in executive function (Lewis, Cools, et al., 2003). The anterior thalamic radiation – the white matter bundle connecting the anterior thalamus with the prefrontal cortex has been implicated in cognitive dysfunction in schizophrenia (Mamah, et al., 2010) and also supports the influence of this fibre tract on cognition.

4.4.3.4 Summary of the limbic nuclei

The limbic nuclei share similar regions of primary connectivity and perform similar functions. Primarily involved in executive function and memory, the AP has been investigated previously in relation to amnesia and is a crucial component of an influential model of information processing (Aggleton & Brown, 1999). The LD has not previously been widely investigated, but due to its projections with the hippocampus the LD can be considered part of the extended hippocampal system mediating memory. The AP appears to be spared by LB pathology, and has not been included in histology studies of PD to date, heavily suggesting

disruption in the frontal circuitry of the limbic loop results in the executive function and memory deficits observed in PD rather than disruption to the nucleus itself.

4.5 Association Nuclei

The association nuclei and the centromedian nuclei are easily visible on MR images and are the most investigated in neurodegenerative disorders. In healthy subjects, examination of the correlations between these nuclei and the cortex have been carried out in one sample to date. Volumetric and metabolic parameters of each nucleus and associated areas of the cortex were obtained and the relationship between them established. Findings followed the known patterns of anatomical connectivity, the mediodorsal nucleus correlated with all cortical regions except the cingulate and occipital areas, the centromedian with temporal, parietal, occipital, dorsolateral and orbital frontal regions and with selected cingulate regions. The pulvinar showed an association with retrosplenial areas, the primary auditory area in the temporal lobe and with selected occipital and parietal areas (Mitelman, et al., 2006).

The association nuclei include the mediodorsal nucleus (MDn) which has strong connectivity with the prefrontal cortex and is involved in attention and executive function processes and the lateral posterior nucleus (LP) and pulivnar (Pu), which share similar reciprocal connectivity with the sensory, motor and visual association areas of the cortex (Nieuwenhuys, et al., 2008b).

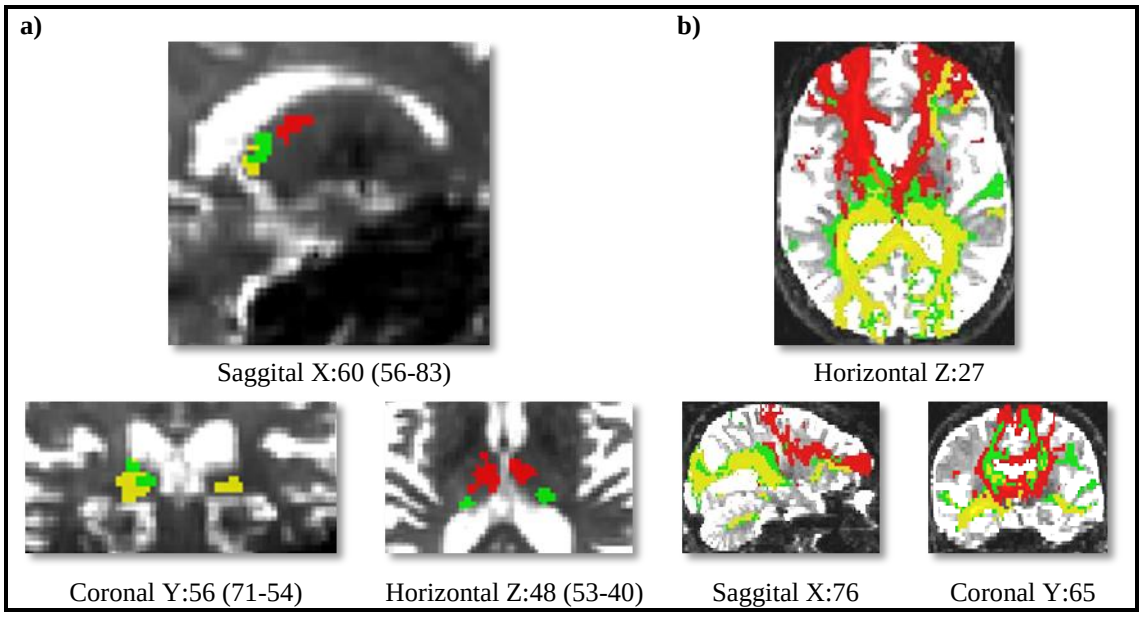


Figure 4-6: Association thalamic nuclei
Anatomical location (**a**) and connectivity (**b**) of the MDn (red), LP (green) and Pu (yellow) nuclei. In each case the section giving the best view of the regions and their connectivity is presented on the top with views in corresponding slices given underneath. X, Y and Z gives co-ordinates in standard (MNI) brain space and

parenthesis the co-ordinates of the first and final slices where the thalamus is visible. MRIcron images in a) are resliced when opened and are thus displayed using neurological convention, the left side of the image corresponds to the left side of the brain. Images in b) are displayed using radiological convention, the left side of the image corresponds to the right side of the brain.

4.5.1 Mediodorsal Nucleus (MDn)

4.5.1.1 Anatomy, location and connectivity

The mediodorsal is one of the largest thalamic nuclei, spanning two thirds of the length of the thalamus. Located posterior to the AP and anterior to the LP nuclei, the primary target of this nucleus is the prefrontal cortex, with afferent connectivity terminating in the anterior cingulate cortex, medial precentral areas and the ventral lateral orbital areas. The MDn is comprised of several smaller nuclei which are easily identified based on their distinct cellular distribution and their independent connectivity with the prefrontal cortex. The magno-cellular division (MD_{mc}) is in the anterior portion of MDn and is made up of large, widely dispersed cell bodies, the axons of which terminate in the dorsolateral regions of the prefrontal cortex. The paravocellular division (MD_{pc}) is located in the lateral regions of MDn and is made up of smaller, more tightly packed cells than the MD_{mc}, and it's axons primarily terminate in the orbitofrontal regions of the cortex (Jones, 2007b). As a whole, the MDn also receives afferent fibres from olfactory areas of the cortex, the lateral orbital cortex and the ventral agranular insular area, regions that, along with the amygdala, comprise the olfactory loop connecting the olfactory cortex, thalamus and neorcortex.

4.5.1.2 Function

The MDn is primarily involved in executive function and working memory processes due to the extensive connectivity with the prefrontal regions and, to a lesser degree, with the temporal, parietal and occipital regions (Buchsbaum et al., 2006). The function of the MDn is mediated by the AP. In patients with thalamic infarctions, when the lesion site included the medial dorsal, midline and/or the intralaminar nuclei, for example, accompanying deficits in executive function were observed. In every case except one there was also significant memory impairment which was worse than the level of executive function impairment. In patients who had both MDn and AP lesions, memory impairment was significantly worse than those who had lesions only in the MDn. Executive function impairment remained approximately the same (van der Werf, et al., 2003) suggesting that while both the MDn and AP play a role in memory and executive function the MDn is an equal contributor to both

domains. When working memory is specifically examined in healthy subjects, reduced working memory performance is directly associated with increased cellular degeneration (measured using mean diffusivity) in the MDn (Piras, Caltagirone, & Spalletta, 2010). The MDn is thought to be primarily involved in the recognition aspects of memory (Aggleton & Brown, 2008) but there is also some evidence to suggest that the MDn is involved in recollection. In otherwise healthy subjects with MDn lesions for example, word recall is significantly impaired alongside executive function deficits despite word recognition remaining intact. This effect was only evident in those with lesions isolated to the lateral MDn (Zoppelt, Koch, Schwarz, & Daum, 2003) which suggests functional dissociation within the MDn.

When both executive function and working memory are examined in conjunction using fMRI the entire MDn region shows a significant increase in activation. In this case MDn segmentation was achieved by isolating all voxels within the thalamus that had strong connectivity with the prefrontal cortex. This method of nuclei identification relies on thalamic-cortico connectivity, suggesting connectivity as well as intact structural integrity of the MDn is essential for correct cognitive processing (Johansen-Berg, et al., 2005). Both sub-regions of the MDn were implicated in the correct execution of executive function tasks, with a strong correlation between the size of MDn sub-regions (medial and lateral) and performance on executive function tasks in healthy subjects (Jakab, Blanc, & Berenyi, 2012).

This has also been demonstrated in Huntington's disease. The regions of the thalamus which had the most connectivity with the prefrontal areas and striatum were the regions that displayed the greatest volume loss. The mediodorsal region was particularly affected and showed the strongest association with executive function and attention measures (Kassubek, et al., 2005).

As attention and executive function deficits are characteristic of Schizophrenia (Lindenmayer, Harvey, Khan, & Kirkpatrick, 2007) the MDn and associated regions of connectivity have been extensively investigated as a neural substrate of this disorder. This has lead to an abundance of imaging research which mostly supports the role of MDn in executive dysfunction. Structural imaging of Schizophrenic patients relative to healthy control subjects has revealed bilateral volume reduction of the MDn independent of any other thalamic regions (Gur, et al., 1998), MDn reduction in conjunction with atrophy of the pulvinar and CM/Pf (Kemether, et al., 2003), volume loss after correction for total thalamic volume (Shimizu, et al., 2008) and volume loss independent of medication levels

(Pakkenberg, 1992). Some studies have also reported volume reduction in the total association nuclei (MDn and Pu) but not in the MDn independently (Byne, et al., 2001). Shape analysis of the whole thalamus has revealed a significant alteration of thalamic shape in Schizophrenia compared to control subjects, postulated to be due to degeneration in the medial-dorsal regions of the thalamus and occurring independently of total volume loss (Qiu, Zhong, et al., 2009).

More recently DTI has been applied to MDn and associated cortical regions in Schizophrenia with results indicating large areas of increased cellular disruption localised to the MDn and prefrontal cortex. Smaller regions of increased diffusivity are also evident in the temporal and parietal lobes (Rose, Chalk, et al., 2006) and diminished anisotropy evident in the white matter of the frontal lobes (Buchsbaum, Buchsbaum, et al., 2006). The frontal and temporal regions of the cortex are the areas to show the fastest rate of degeneration (Mathalon, Sullivan, Lim, & Pfefferbaum, 2001), and have a strong correlation with memory and executive function in this cohort (Baare, et al., 1999). Cortical degeneration in these areas also results in disrupted connectivity and the subsequent cellular degeneration of the MDn. Gross structural changes show the same result, volume reduction in the prefrontal and orbito-frontal cortex is correlated with the level of volume reduction in the mediodorsal regions of the thalamus (Kim, et al., 2007).

These changes in associated subcortical and cortical regions suggest a deficit in the thalamic-prefrontal circuitry must also be present. In one of the few studies to investigate this in Schizophrenia, Kito et al., (2009) used diffusion tensor imaging to examine the structural integrity of the anterio thalamic peduncle – the neuronal tract which runs from the MDn to the dorsolateral prefrontal cortex via the anterior limb of the internal capsule. There was a significant reduction in cross-sectional area of the tract, suggesting some disruption to the frontal-striatal-thalamic pathway. When this same tract was investigated in a similar study, tract integrity was also found to be significantly disrupted and overall length of the tract was shorter in schizophrenia patients compared to healthy control subjects, (Buchsbaum et al., 2006).

Interpretation of the association between degeneration and cognition is limited by studies that have carried out cognitive and behavioral analysis. Only two of the above studies examined neuropsychiatric symptoms, with one showing no association between structure and function (Kito, et al., 2009) and the other reporting a high correlation between MDn regions and both negative symptoms and antipsychotic medication dose in Schizophrenia (Kim, et

al., 2007). Qiu et al., (2009) was the only study to report an association between the thalamus and cognition, showing that their cohort of Schizophrenic patients had poorer performance on executive function and spatial working memory tasks compared to the control subjects and a significant correlation between both cognitive measures and the compressed shape of the thalamus in the medial dorsal region.

The MDn region also shows an association with executive function in an Alzheimer's disease sample, exhibiting significantly reduced volume in patients relative to control subjects. The observed volume reduction was thought to be primarily due to degeneration of the medial-dorsal regions based on the fact it is these regions most targeted by LB pathology (de Jong, et al., 2008). More robust analysis of the thalamic shape in AD also supports this result, showing that atrophy in the medial-dorsal regions of the thalamus correspond to decreases in the cellular integrity of the MDn region (Zarei, et al., 2009). MDn shape changes are possibly only evident when cognitive decline is advanced however. When both dementia and pre-AD mild cognitive impairment patients were examined, the medial thalamus of the MCI group was not any different to that of the control group and only showed a significant shape change in the AD group (Qiu, Fennema-Notestine, et al., 2009).

4.5.1.3 Cognitive deficits in PD associated with nuclei dysfunction

Clinically, PD patients show a range of attention, executive function and memory deficits (Janvin, et al., 2003). Cognitive deficits, especially those in the executive function domain are associated with changes in frontal lobes and fronto-striatal circuitry (Lewis, Dove, et al., 2003), indicating a mediation role for the MDn and its associated connectivity. In an fMRI paradigm using a motor task for example, performance was significantly worse in the task which required the patients to pay attention in order to successfully complete it. Patients were either required to pay attention in order to perform the task or had previously over learned the task so it was automatic. Furthermore, although associated with activation of prefrontal and supplementary motor areas of the cortex in controls, patients failed to show the expected increase in prefrontal activation during the attention task (Rowe, et al., 2002).

The neurocorrelates of executive function are similarly affected, Parkinson's patients with and without executive function impairments who were well matched on all other areas of cognitive function were examined using a working memory task which was designed to assess dorsolateral and ventrolateral executive systems. In the subgroup with executive function deficits, task performance was significantly associated with reduced activity in the

prefrontal regions while prefrontal activity remained normal in the group without executive impairment (Lewis, Cools, et al., 2003).

4.5.1.4 Associated areas of PD pathology and degeneration

Surprisingly, the MDn itself has rarely been examined in PD, with only a handful of autopsy studies and one imaging study isolating this nucleus. Autopsy studies have shown low levels of LB pathology (Braak & Braak, 2000; Rub, Del Tredici, Del Turco, & Braak, 2002) but no neuronal (Halliday, 2009) or volume (Henderson, et al., 2000b) loss in the MDn. As none of the patients in any of these studies had cognitive impairment however, this could just reiterate that the MDn is not a big factor in motor dysfunction in PD. Only one imaging study to date (Li, et al., 2010) specifically isolated the thalamus and identified the MDn as showing change in PD using voxel based morphometry. A small group of PD patients with and without depression were recruited and the whole thalamus identified *a priori* as a region of interest which could potentially be implicated in depressed PD patients. Although there were no changes in mean diffusivity in any regions of the thalamus, FA measures did show a significant decrease in the medial-dorsal region of the depressed PD group compared to the non-depressed group. All patients were in early to mid-stage of disease and although in depth cognitive analysis was not conducted, their MMSE scores indicate relatively intact cognition (MMSE ~ 29.3). The authors, of course conclude that the MDn is a neurocorrelate of depression, which is possible but it should also be noted that when depression is screened for and specifically excluded as is done in our own cohort (Melzer, et al., 2011b) atrophy in the medial thalamic region remains a significant predictor of global cognitive measures in PD.

4.5.2 Pulvinar Nucleus (Pu)

4.5.2.1 Anatomy, location and connectivity

The pulvinar is the largest thalamic nucleus and makes up one quarter of the thalamus (Letinic & Kostovic, 1997). The pulvinar is comprised of four distinct components, the anterior pulvinar (Jones, 2007b) which has projections to the somatosensory cortex in the parietal lobe (Hirsch, 2000), the inferior pulvinar which has primary connectivity with the visual cortex, the medial pulvinar, linked to the temporal and parietal lobes and the lateral pulvinar which has afferent terminations in visual, temporal and parietal cortices (Highley, Walker, Crow, Esiri, & Harrison, 2003). The cellular make up of these regions is distinct and the subdivisions easily identified. The medial division has small and widely dispersed cells, the

lateral, cells of a similar size, broken up by the fibres of the corticotectal tract that runs through this region. The inferior nucleus has densely packed cells, with most cells relatively small but some large cells also scattered throughout. The anterior division has the smallest and most widely dispersed cells of all those in the pulvinar. For the purpose of this research all components of the pulvinar were combined into one nucleus. The main input to the pulvinar is from the visuomotor regions of the brain, specifically the pretectum and the superior colliculus. Corticothalamic fibers are also received from the striate and extrastriate cortex, terminating in all regions of the pulvinar (Jones, 2007b). In addition, the pulvinar is interconnected with several subcortical structures including the superior colliculus, other thalamic nuclei and caudate nucleus along with the primary and secondary visual areas, visual inferotemporal areas, parietal association areas and prefrontal areas which mediate visual information processing and visual spatial attention (Leh, Chakravarty, & Ptito, 2008).

4.5.1 Lateral Posterior Nucleus (LP)

4.5.1.1 Anatomy, location and connectivity

The lateral posterior nucleus (LP) is comprised of a cluster of densely packed medium sized cells. Located dorsally adjacent to the pulvinar, the projections of the LP terminate in the same cortical areas as the Pu, with the projections of the LP rostal to those of the anterior pulvinar (Nieuwenhuys, et al., 2008b). Although definition of the subdivision between the LP and Pu has not always been consistent (Jones, 2007b) and MRI studies often combine the two nuclei (Nieuwenhuys, et al., 2008b) we have considered the LP and Pu separately in this study, with each segmented from the whole thalamus independently. As they essentially influence the same functions however, their functionality will be reviewed together.

4.5.2 Combined LP-Pu Complex

4.5.2.1 Function

The pulvinar is most recognised for its visual influence (Jones, 2007b). Specifically, the interpretation of visual stimuli and visuospatial attention is served by the pulvinar. Given all the objects present in the human visual field at any one time, the goal of the visuospatial system is to only use the information from the target object and ignore all other irrelevant stimuli. Several fMRI studies conducted on healthy control subjects have further expanded our understanding of the Pu. The Pu shows increased activation when participants are

required to attend to a target compared to a non target stimuli during a fMRI procedure (Buchsbaum et al., 2006). The Pu connectivity with the extrastriate cortex, a region of the occipital lobe adjacent to the primary visual field appears to be primarily responsible for the detection of visual stimuli. Hierarchically organised, specific areas of this cortex are associated with the perception of colour, movement, shape, faces and spatial locations. Spatial location of objects appears to be the main function of this region of the occipital lobe as increased activation is evident in this region during tasks requiring detection of location rather than face matching in healthy control subjects (Hazxby, et al., 1994).

This response is not elicited just from the presentation of visual stimuli, as the Pu only shows activation (Kastner, et al., 2004) or increased glucose uptake (a measure of increased activation) (LaBerge & Buchsbaum, 1990) if the participant is instructed to pay attention to the stimulus. No activation is evident under passive conditions when the subject is instructed to remain fixated on a central point whilst stimuli are presented. Attending to visuospatial information proves impossible when the Pu is lesioned. Significant visuospatial hemineglect is evident in lesion patients as they are unable to direct attention to the visual hemifield contralateral to the lesion site (Karnath, Himmelbach, & Rorden, 2002).

Similar to the mediodorsal nucleus, the pulvinar has also been implicated in Schizophrenia. Structural MRI analysis has shown the Pu to be significantly reduced in patients relative to control subjects (Byne, et al., 2001), even after correction for total brain volume (Kemether, et al., 2003) and, in both cases in the absence of wider thalamic degeneration. Shape analysis also revealed inward deformation of the posterior region of the thalamus in schizophrenic subjects relative to controls (Harms, et al., 2007).

The combined function of the LP and Pu, examined together as 'posterior thalamic nuclei' have a significant effect on visual spatial function and perception. In patients with lesions restricted to the posterior region of the thalamus a significant deficit in several spatial and perception tasks is evident. Visual discrimination, especially, was lower in those with posterior thalamic regions compared to those with lesions in frontal or straital regions, suggesting the LP and Pu are strong components of intact visual/spatial function. There was no association between visuospatial function and measures of executive function in this cohort. The posterior nuclei do not appear to be mediated by executive function process when contributing to intact visual spatial function (Ricker & Millis, 1996).

The pulvinar could also mediate memory function as significant volume reduction of the temporal lobe, one of the main regions of connectivity with the pulvinar is evident in

schizophrenic patients. Volume reduction of the temporal lobe is consistently reported, of 51 cohorts, 61% showed volume reduction, (see Shenton, Dickey, Frumin & McCarley, (2001) for review). The same review also reports that of MRI studies that examined the parietal lobe, 60% found a reduction in volume. Studies regarding the occipital lobe are not as clear however as only 44% report occipital volume differences in Schizophrenia, and this from a sample size of only 9 studies that have examined the occipital lobe.

In regards to the influence on cognitive function, structural MR studies tend to report no association between cognition and the temporal or occipital lobes (Seidman, et al., 1994), with a recent fMRI meta-analysis also reporting no difference in activation of the temporal lobes during a cognitive processing task between controls and Schizophrenic patients (Davidson & Heinrichs, 2003).

The pulvinar nucleus itself shows a strong association with cognition however and may be more involved in cognitive dysfunction than the connectivity of associated cortical regions. In patients with a form of dementia called subcortical ischemic dementia (SVD) that primarily exhibits deficits in executive function and memory (Erkinjuntti, 2003), blood flow in the pulvinar is significantly reduced compared to controls. Blood flow was significantly associated with cognitive score (MMSE) in the left frontal region, suggesting connectivity disruption between the pulvinar and frontal cortex (Kato, et al., 2008). The pulvinar is also associated with more general measures of cognition, Coscia (2009) reports significant shape changes in the pulvinar region of the thalamus which are associated with global measures of cognition and language, motor and executive functions. Shape change of the pulvinar is also associated with memory, Harms, et al., (2007) showed a weak correlation between shape change in the thalamus that was thought to correspond to pulvinar degeneration and working memory, while Andrews, et al., (2006) reports pulvinar activation during a working memory task in Schizophrenia.

4.5.2.2 Cognitive deficits in PD associated with nuclei dysfunction

Clinically, PD patients exhibit a significant degree of visuospatial deficits (Cronin-Golomb & Braun, 1997; Owen, et al., 1993), even in the early stages in some cases (Janvin, et al., 2003). Manifesting as an array of visual perceptual deficits or visual spatial deficits, visual impairment heavily impacts on patient daily function and caregiver burden. Visual perceptual deficits include: detection of colour, motion and deficits in facial scanning – leading to problems with the recognition of emotion. Visual spatial deficits arise from difficulty in

perceiving the spatial relationship among stimuli, presenting as difficulty in tasks such as judging the relationship between a coffee cup and table when trying to place the cup down, or between a moving vehicle and one that is stationary at a red light up ahead when preparing to stop (Seichepine, et al., 2011). A range of neuropsychological tests can measure the degree to which patients are affected. The fragmented letter subtests derived from the visual Object Space and Perception (VOSP) battery can be used to assess visuoperception deficits in patients while the Judgement of Line orientation (JLO) can be used to assess visuospatial deficits.

4.5.2.3 Areas of pathology and degeneration in PD associated with the LP and Pu

Neither the LP or Pu exhibit extensive pathology in PD, the pulvinar nucleus exhibits isolated LB's in the anterior and medial subregions with slightly higher LB or LN infiltration evident in the LP (Rub, Del Tredici, Schultz, et al., 2002). Despite this, the pulvinar nucleus has shown a significant degree of grey matter loss in PD-D relative to PD after inclusion of clinical movement characteristics in our cohort (Melzer, et al., 2011b), suggesting a significant role in cognitive dysfunction in PD.

4.5.2.4 Summary of the association nuclei

The association nuclei are easily identifiable on MR images, and are frequently examined in neurodegenerative disease, most commonly in Schizophrenia (Byne, et al., 2001; Kemether, et al., 2003). Although several studies report volume reduction of these nuclei, normally in the absence of wider thalamic reduction – very few have carried out any investigation into the effect that this has on cognition. Primarily connected to temporal and parietal lobes (Highley, et al., 2003), with the MDn also projecting to frontal regions (Buchsbaum, Schoenknecht, et al., 2006), these nuclei could be involved in memory, attention, executive function and visual perception in PD. The LP and Pu do not exhibit extensive pathology at autopsy (Rub, Del Tredici, Schultz, et al., 2002), but regions of primary connectivity such as the temporal (Summerfield, et al., 2005) and frontal lobes (Karagulle Kendi, et al., 2008), do show significant change in PD subjects. Disruption in the thalamic-cortico, especially the frontal circuitry could therefore be a factor in cognitive dysfunction in PD.

4.6 Sensory Relay Nuclei

The sensory relay nuclei have projections which ascend to the primary sensory fields of the neocortex (Nieuwenhuys, et al., 2008b) and are involved in somatosensory information such as touch, vision and auditory function. New evidence suggests that it is the interaction between our different senses, occurring in the higher association areas of the neocortex that mainly aids our interpretation of the environment (Kayser & Logothetis, 2007). A deficit in any of the association cortices could therefore cause a myriad of symptoms which affect cognitive processing and function. The sensory relay nuclei include the medial (MGN) and lateral (LGN) geniculate nuclei and the ventral posterior nucleus (VP). The medial and lateral geniculate nuclei are involved in auditory and visual processing. Reciprocal connections from these regions preferentially terminate in respective association areas of the temporal and occipital cortex (Nieuwenhuys, et al., 2008b). Although intact visual and auditory functioning will moderate cognition in PD, due to the similar connectivity of these nuclei to the pulvinar and the limited influence over other aspects of function, they will not be included for examination in this cohort. Located ventral and either medial or lateral to the main dorsal thalamus, the MGN and LGN are automatically excluded from the dorsal thalamus during the automatic segmentation procedure we have applied here (Patenaude, 2007).

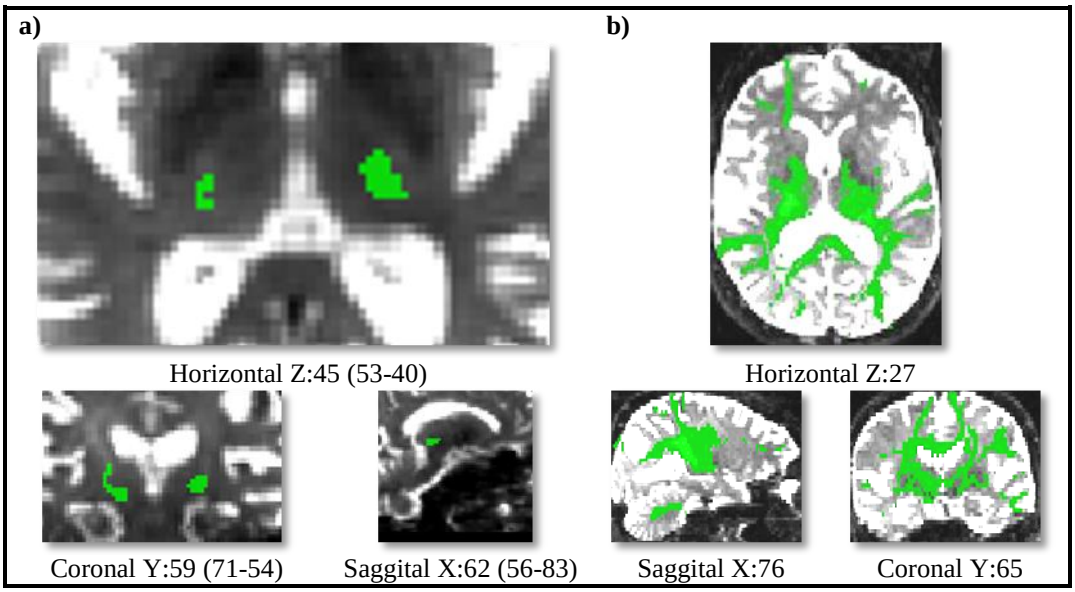


Figure 4-7: Sensory thalamic nucleus
Anatomical location (**a**) and connectivity (**b**) of LP nuclei. In each case the section giving the best view of the regions and their connectivity is presented on the top with views in corresponding slices given underneath. X, Y and Z gives co-ordinates in standard (MNI) brain space and parenthesis the co-ordinates of the first and final slices where the thalamus is visible. MRIcron images in a) are resliced when opened and are thus displayed using neurological convention, the left side of the image corresponds to the left side of the brain. Images in b) are displayed using radiological convention, the left side of the image corresponds to the right side of the brain.

4.6.1 Ventral Posterior Nucleus (VP)

4.6.1.1 Anatomy, location and connectivity

The main projections of the ventral posterior nucleus are to the somatosensory cortex, terminations primarily end in the primary sensory cortex and, to a lesser degree in the secondary sensory cortex. There are two main inputs to the VP, which work to convey sensation and communicate information relating to pain, temperature and touch of the body (Bhatnagar, 2002). The first input is from the trigeminal lemniscus which communicates information from the head, trunk and oral structures and terminates in the medial region of the ventral posterior nucleus (VPM). The dorsal columns-lemnisal pathway receives input from the contralateral limbs and trunk and terminates in the lateral region of the ventral posterior nucleus (VP). The two nuclei divisions are easily distinguishable, separated by the white matter of the arcuate lamella and are also easily identified by their cellular make up. The VPM is composed of a high density of small closely packed neurons whilst the VP is an arrangement of larger cell clusters (Jones, 2007b). Here we have combined the ventral lateral and medial components of the ventral posterior nucleus and considered them as one ventral posterior complex.

4.6.1.2 Function

The VP primarily appears to be involved in pain. The VP is involved in patients with central pain syndrome, a disease of the central nervous system involving neuronal systems which communicate the sensation of touch in the somatomotor system. Symptoms include pain, abnormal regulation of blood flow, increased fluid in the skin and movement dysfunction in the absence of any other neurological conditions (Janig & Baron, 2002). The VP shows increased neuronal firing in the areas of the VP which represent the body part where the patient experiences pain (Lenz, 1992). Evidence for the role of the sensory nuclei also comes from lesion studies in dystonia – a neurological condition which presents as twisting and repetitive movements or abnormal postures of the body (Fahn, 1988). Lesions of the ventral posterior nucleus are found to cause dystonic symptoms which can be differentiated by the region of the VP that is affected. Lesions in the lateral VP for example cause dystonic spasms - sustained muscle contraction causing prolonged spasm, while lesions encompassing both the medial as well as lateral VP result in myoclonic dystonia – rapid jerking movements (See Deleu, Lagopoulos, & Louon, (2000) for review).

4.6.1.3 Sensory deficits in PD associated with nuclei dysfunction

Not surprisingly, pain is significantly associated with symptoms of dystonia in PD. Compared to healthy controls, patients with PD report significantly greater levels of pain, associated with the degree of dystonic symptoms (Defazio, et al., 2008). Sensory complaints that are unrelated to motor symptoms have also been described in PD patients and include aching, numbness, tingling, burning and vibrating sensations in an extremity (Snider, Fahn, Isgreen, & Cote, 1976).

4.6.1.4 PD Pathology

There is some evidence to suggest that disruption within the somatosensory cortex causes the experience of painful sensations in PD patients. One controlled PET study has been carried out using PD patients and control subjects to examine differences in cerebral activity during simulation of pain receptors. Neither group were experiencing pain symptoms before the study, enabling the same level of pain stimuli to be administered during the scanning procedure. Pain threshold was lower in patients than controls and corresponded to higher activation of somatosensory cortex pathways. Pain threshold returned to normal ranges after administration of levodopa in the patients but did had not effect on the pain symptoms in controls, suggesting a primary role for the degeneration of dopaminergic neurons in the increased perception of pain in PD patients (Brefel-Courbon, et al., 2005). In previous studies which have examined pathology of thalamic nuclei (Halliday, 2009; Henderson, et al., 2000a), the VP region was not included so it is difficult to determine the level of degeneration that occurs in this region and the consequent effect this has on the sensory deficits seen in PD.

4.6.1.5 Summary of the sensory nucleus

The VP nucleus is primarily involved in pain perception (Lenz, 1992), with some evidence also suggesting an influence over the movement symptom of dystonia (Defazio, et al., 2008). The VP has not been previously examined for level of LB infiltration in PD, making it difficult to gauge the level of degeneration that occurs in this region. A single imaging study has shown the somatosensory cortex – the region of primary association with the VP to be significantly affected during the perception of pain in PD patients (Brefel-Courbon, et al., 2005) however, suggesting degeneration of connectivity between this thalamic region and the cortex underlies sensory disruption in PD.

4.7 Intralaminar Nuclei

The intralaminar nuclei have traditionally been considered non-specific nuclei, nuclei that have such diffuse cortical projections that they influence multiple behaviours. Modern anatomical tracing techniques have recently revealed the CM/Pf complex projects preferentially to particular cortical areas however, with only slight overlap in the projection fields of adjacent nuclei (Groenewegen & Berendse, 1994). Similar to the function of the pulvinar, there is also evidence to suggest CM/Pf involvement in attention tasks in healthy subjects. The CM/Pf region was the only thalamic region to show an increase in blood flow during an attention task compared to resting state in healthy control subjects (Kinomura, Larsson, Gulyas, & Roland, 1996).

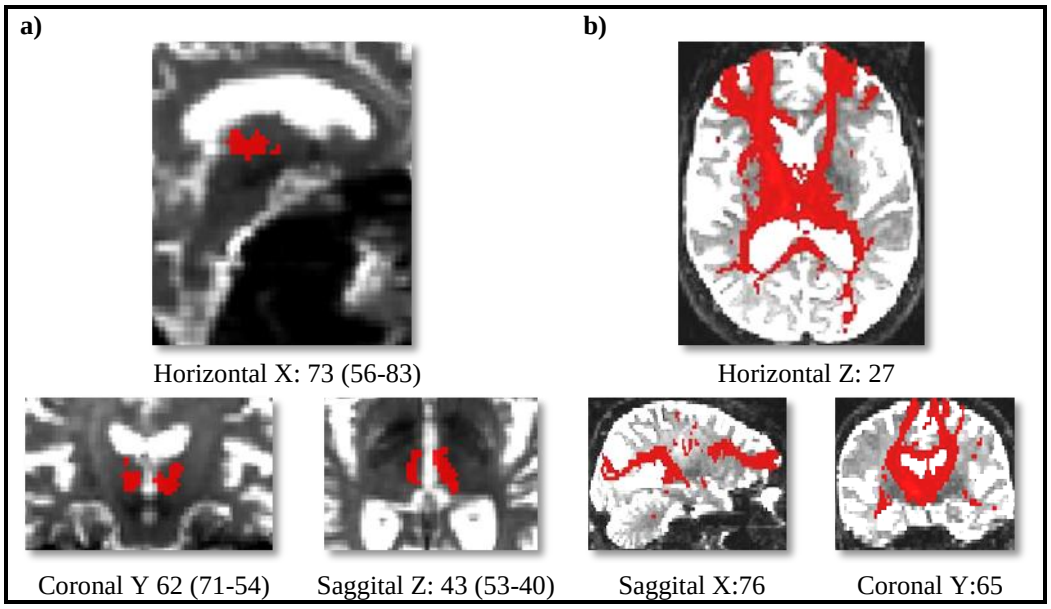


Figure 4-8: Non-Specific thalamic nucleus
Anatomical location (**a**) and connectivity (**b**) of the CM/Pf nucleus. In each case the section presenting the best view of the regions and their connectivity is presented on the top with views in corresponding slices presented underneath. X, Y and Z give coordinates in standard (MNI) brain space and parenthesis the co-ordinates of the first and final slices where the thalamus is visible. MRIcron images in a) are resliced when opened and are thus displayed using neurological convention, the left side of the image corresponds to the left side of the brain. Images in b) are displayed using radiological convention, the left side of the image corresponds to the right side of the brain.

4.7.1 Centromedian – Parafascicular Complex (CM/Pf)

4.7.1.1 Anatomy, location and connectivity

For the purpose of this research the centromedian (CM) and parafascicular (Pf) nuclei were combined and analysed together. These nuclei are found within the posterior region of the internal medullary lamina and are easily differentiated by their cellular distributions as the

parafascicular nucleus is made up of dense cells in the posterior region of the internal medullary lamina while the centre median nucleus extends laterally from the parafascicular nucleus and is comprised of small, closely packed cells. There are widespread connections from the intralaminar nuclei throughout the cortex, and although a few intralaminar cells project to every cortical area, the main projections of the CM/Pf complex are to the striatum. In regards to cortical connectivity, the majority of CM cells project to medial and basal areas of the cortex while a smaller number project to the primary motor cortex. The Pf projects to deeply placed areas around the rhinal sulcus and to the cingulate gyrus. In its entirety, the complex receives afferent fibres from the basal ganglia, midbrain, cerebellum and spinal cord with the CM alone receiving projections from the globus pallidus, motor cortex and limbic cortex (Jones, 2007b).

4.7.1.2 Function

To date, two studies have specifically set out to examine the CM/Pf complex, both of which isolated both the CM/Pf and the association nuclei in Schizophrenia. Contrasting results are reported, Byne et al., (2002) shows volume and neuronal reduction in the MDn and Pu nuclei, but not in the CM/Pf, and Kemether, et al., (2003) shows volume loss all three thalamic nuclei. Both the centromedian and parafascicular nuclei do also independently show reduction in those classified as 'severely disabled' after a traumatic brain injury when compared to control participants (Maxwell, MacKinnon, Smith, McIntosh, & Graham, 2006). This could indicate that volume loss is related to degeneration of cortico-thalamic pathways. Significant damage to the cerebral cortex could result in neuronal loss in fibre pathways that connect to subcortical regions, for example, an idea that appears to be supported by this data. The parafascicular complex showed the highest reduction, suggesting that this region has weaker connections to the cortex than the centromedian nuclei.

4.7.1.3 Cognitive deficits in PD associated with nuclei dysfunction

Deficits in executive function, attention and memory in PD associated with the association nuclei have already been reported above. These cognitive domains could also be influenced by the CM/Pf due to the association between this aspect of cognition and the anterior cingulate and dorsolateral prefrontal areas (Mitelman, et al., 2006).

4.7.1.4 PD Pathology

Lewy pathology heavily infiltrates the CM, and to a lesser degree the Pf nucleus in PD (Rub, Del Tredici, Del Turco, et al., 2002), pathology is at a level only slightly below that of the components of the limbic loop (Halliday, 2009). Both regions also show a heavy degree of neuronal loss, and evidence of atrophy in the Pf nucleus (Henderson, et al., 2000a). The CM/Pf complex has also been implicated in PD patients without cognitive impairment. In an imaging study which simultaneously examined volume and shape of the thalamus, differential reduction of the CM/Pf complex was reported. There was a significant change in thalamic shape, thought to be due to CM/Pf degeneration without an overall reduction in volume of the whole thalamus (McKeown, et al., 2008). There is also some evidence to suggest that degeneration in the intralaminar areas contributes to PD dementia, there is an association between the central lateral (CL) nucleus and PD-D. The CL is a small intralaminar nucleus located adjacent to the CM. Volume loss in this region is more acute in PD-D than in PD without dementia (Brooks & Halliday, 2009). As the CL shares similar reciprocal connectivity as the main CM/Pf complex, it is likely that this region will be included with the wider CM/Pf nuclei when the thalamus is segmented and may influence the relationship between CM/Pf and cognition in our sample.

4.7.1.5 Summary of the intralaminar complex

Although the intralaminar region has traditionally been considered a 'non-specific' nucleus (Jones, 2007b) evidence from schizophrenia samples (Kemether, et al., 2003) suggests this region has influence on some behavioural and cognitive functions. There is extensive pathology in the intralaminar nuclei in PD (Brooks & Halliday, 2009; Rub, Del Tredici, Del Turco, et al., 2002) and degeneration in this region could influence the cognitive symptoms of Parkinson's disease.

4.8 Summary

In other neurodegenerative disorders such as Alzheimer's disease and Schizophrenia there is some evidence to suggest that neuronal integrity (Buchsbaum, Buchsbaum, et al., 2006; Rose, Chalk, et al., 2006; Zarei, et al., 2009), neuronal loss (Byne, et al., 2006; Young, et al., 2000) or gross volume reduction (de Jong, et al., 2008; Gilbert, et al., 2001; Gur, et al., 1998; Hazlett, et al., 1999; Kemether, et al., 2003; Pakkenberg, 1992; Shimizu, et al., 2008) in individual thalamic regions has an association with cognition. In PD, histology studies have

reported differential infiltration of Lewy pathology in thalamic nuclei (Halliday, 2009; Henderson, et al., 2000b), in some cases also reporting significant neuronal and volume loss which corresponds to the degree of LB burden (Henderson, et al., 2000a).

Our group has previously provided significant justification for the current thesis as, using robust methodology and advanced neuropsychological testing we have shown a strong association between specific domains of cognitive dysfunction and grey matter loss in the pulvinar nuclei and significant grey matter loss in cortical areas known to be reciprocally connected with thalamic nuclei (Melzer, et al., 2011b). We have also shown disruption in the connectivity between anterior thalamic regions and the neocortex (Melzer, et al., 2011a). The individual components of the thalamus have not yet been isolated a priori and examined in relation to cognition however. Given the extensive connectiviety between the thalamus and the neocortex, and thalamic involvement in cognitive symptoms, this suggests there still remains a significant gap in the research. The thalamic nuclei are hypothesised to be a good indicator of current cognitive dysfunction in PD, and a tool for the monitoring of disease progression in the future.

Chapter 5. METHODOLOGY

5.1 Objectives

The participants in this thesis are a convenience sample obtained from a wider group of participants who are involved in ongoing research at the New Zealand Brain Research Institute, (NZBRI: formally the Van der Veer Institute) Christchurch. This chapter describes the methodology surrounding the recruitment and examination of the participants in the wider research. A brief background surrounding the relevant aspects of MR and DT imaging and the wider image acquisition procedure is also described. This thesis employed several imaging techniques, and as such, the elimination of some subjects from aspects of the study was necessary. This was primarily due to image artefacts or other abnormalities that were identified during the scanning procedure. Further methodological details for each of these subsets will thus be described in the relevant subsequent chapters.

5.2 Participants

A sample of one hundred and forty two patients who were part of an ongoing longitudinal study at the NZBRI who had undergone neuropsychological testing on or before 25 March 2010 were included in the initial cohort. Parkinson's disease had previously been diagnosed in these individuals based on the UK Parkinson's disease society's criteria for idiopathic PD (Hughes, et al. 1992) by an experienced clinician (T.A). Participants were excluded from the study if they showed any of the following disorders: atypical Parkinson disorder; other central nervous system disorder; a prior learning disability; a major psychiatric or medical illness in the previous 6 months or a previous history of neurological conditions including: moderate-severe head injury; stroke and vascular dementia. All patients were tested in an "ON" state where most patients were receiving an anticholinergic agent or L-dopa in addition to another dopamine agonist.

From this initial sample, further exclusion criteria specific to this thesis was necessary and is outlined in *Figure 5-1*. Patients who had not yet undergone or had not completed the entire DT and MR scanning procedure on or before 13 March 2010 (scanning began for this sample on the 26 May 2007) were not able to be included in the study. Patients with movement artefacts or other brain abnormalities were also excluded. Control subjects were mainly excluded due to cognitive dysfunction or abnormal image artefacts.

The healthy control group consisted of thirty eight individuals who were part of the volunteer database held at the NZBRI. The participants on this database are recruited through community advertising and matched to the patient group on mean age, sex ratio and education level after recruitment by a trained examiner (L.L.) and are only contacted for those studies where their characteristics match the age, sex and education levels of the participant group of that study. Participants were required to meet the same inclusion criteria as the patients and were additionally excluded if they met NZBRI criteria for mild cognitive impairment (*Section 5.2.2*), or had a MoCA score below 26, the recommended cutoff for cognitive impairment based on normative data (Nasreddine, et al., 2005). Some control participants were also excluded after the MR imaging procedure revealed brain abnormalities such as cysts or lesions. Informed consent was obtained for all participants after they had read through an overview of the research and understood what it was they were required to do in order to be included in the study. Where additional consent was required, informed consent was provided by the patients' significant other. Volunteers were compensated for their time and travel requirements with vouchers of $30 for each neuropsychological testing session and $20 for the MRI scan. Ethics approval was obtained by the local Ethics Committee, New Zealand Ministry of Health.

5.2.1 Recruitment Procedure

Parkinson's disease patients are recruited from the specialist movement disorders clinic at the New Zealand Brain Research Institute. The eligibility of the patient for any upcoming research is assessed and an information sheet and outline of the study they are most eligible for is posted to the patient. Should they wish to participate, patients are asked to phone the institute. For this thesis the patient sample was obtained from two ongoing studies, the PD study and the biomarkers study. For both of these wider studies the recruitment procedure was the same. It was desirable that the patients completed all components of the study within one month, so they were generally booked in for all appointments simultaneously. Two neuropsychological test sessions, one eye movement examination and one MRI scan were required. During the eye movement appointment the patient was also required to make sure the individual acting as their significant other was available for a separate interview. Eye movements were assessed by another examiner (C.G. or T.P.). This enabled the psychometrician that examines the patient to be also able to see the patients' significant other, maximising the opportunity to gain detailed information about the patient.

For the studies within this thesis, all patients from the PD study and the biomarkers study were initially included and systematically excluded if they had not completed all components of the research at the end of the first year or did not meet the individual requirements for this thesis. All cognitive, demographic, clinical and behavioural data was initially obtained from patient files along with the information from the significant other. Eye movement information was not included.

All healthy controls that were part of the wider PD study were included in this research. The final sample was 92 PD patients (PD-D = 17; PD-MCI = 19, PD-N = 56) and 25 control subjects.

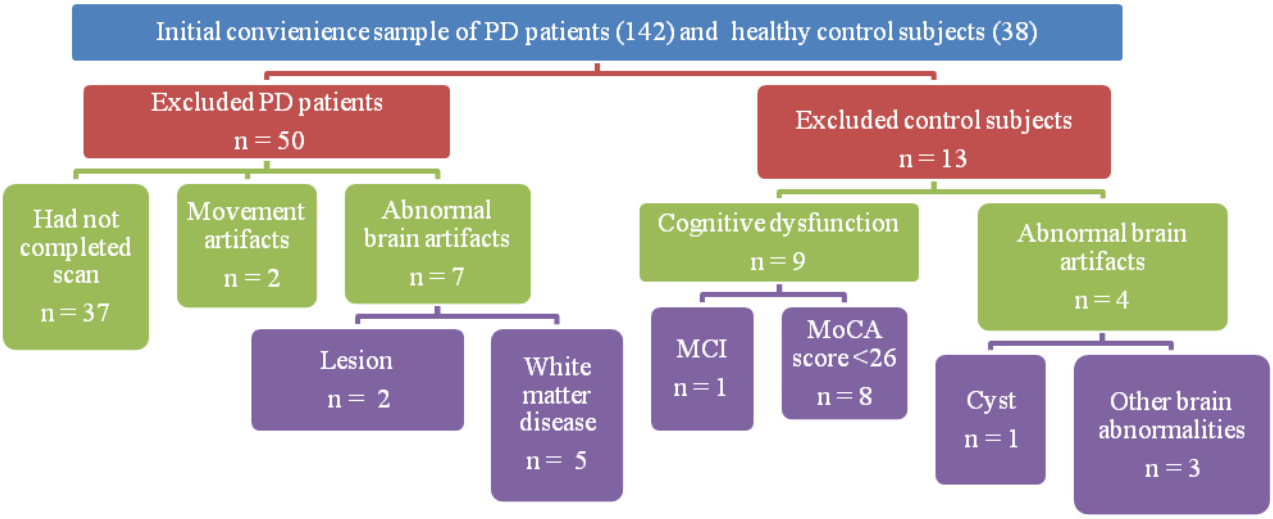


Figure 5-1: Subjects that were excluded from the initial sample.
The number of subjects excluded at each step, the final sample was 92 PD patients (PD-D = 17; PD-MCI = 19, PD-N = 56) and 25 control subjects.

5.3 Neuropsychological testing

Cognitive examination was completed in two sessions of up to two hours each. The first Neuropsychological Inventory 1 (NP1) was administered in the first session and the second Neuropsychological Inventory 2 (NP2) in the third session. Both NP inventories consisted of a series of cognitive tests which, in their entirety examined all cognitive domains recommended by the Movement Disorder Society task force in the diagnosis of mild cognitive impairment and dementia (Emre, et al., Dubois, et al., 2007; 2007) in Parkinson's disease: learning and memory; executive function; language; visuospatial function and attention.

5.3.1 Demographic and Clinical Information collected for all participants

Demographic information was collected from database information and medical files for each participant and included age, sex, handedness and years of education. Handedness was defined as the hand an individual wrote with, not the hand that is used predominately in everyday tasks. Education was defined as the number of years in a formative institution, including primary school.

5.3.1.1 Older Person's Mood Scale: Brief 15-item version

For the control participants, the level of depressive symptoms was the only clinical information collected and was measured using the brief 15-item version of the Geriatric Depression Scale (GDS: Yesavage, et al., 1982). As major depression had already been excluded in this cohort the administration of the GDS was applied only to give an indication of sub-threshold depressive symptoms and did not result in a diagnosis of depression. The GDS was completed during session 1 and initially asked the participant 4 questions about their life for which they are to respond either 'yes' or 'no'. The questions include items such as 'are you basically satisfied with your life?' and 'do you prefer to stay at home rather than going out to do new things?' If the participant answers 'yes' to any of the first four questions this indicates depression is present and the rest of the scale is administered to gain more insight into the level of depressive symptoms. Items 5-15 are also 'yes' or 'no' questions and include items such as 'do you often feel helpless?' and 'do you feel that life is empty?' Participants are assigned one point for every 'yes' answer and given a score out of 15. The GDS has been validated against both the Hamilton Rating Scale for Depression (HRS-D: Hamilton, 1960) and the Zung Self-Rating Depression Scale (SDS: Zung, 1965) and found to be a reliable and valid self-rating screening scale for the elderly (Yesavage, et al., 1982).

5.3.2 Clinical Information collected for patients only

The same demographic information was collected for the patient group. In addition, the clinical information pertaining to disease duration, disease severity and disease stage was collected from patient files and The Unified Parkinson's Disease Rating Scale (UPDRS: Fahn, Elton, & Committee., 1987). Disease duration was defined as the time from diagnosis, not the number of years that symptoms had been present. Disease severity was measured using Part III – motor examination of the UPDRS and disease stage with the Hoehn and Yahr (Hoehn & Yahr, 1967) which forms part V of the UPDRS.

5.3.2.1 The Unified Parkinson's Disease Rating Scale

The UPDRS is administered during session two. For the purposes of this thesis only the scores for Part III and Part V were collected from patient files. In its entirety the UPDRS allows for examination of the patient by a trained examiner across several different functions and also includes a historical report of mental function and activities of daily living based on patient self-report. Parts I, II and III contain 44 questions which are each measured on a 5 point scale (0-4). Part I measures mentation, behaviour and mood through the examination of intellectual impairment, thought disorder, motivation and depression. Part II measures the patients' level of activities of daily living including their speech, swallowing, handwriting, dressing and hygiene. Part III is described below and examines motor function. Part IV consists of 11 questions about any complications the patient may be experiencing due to therapy such as dyskinesia-duration, dyskinesia-pain and nausea or vomiting using a subjective rating scale which ranges from 0 to 23. Part V is the modified version of the Hoehn and Yahr exam. For each subsection the patient is assigned a total score based on the rating of severity (where a higher score indicates more severity) and a total UPDRS score (range 0 – 176) is the combined sum of parts I – IV. Although the UPDRS is a subjective rating scale, the inter-rater reliability is high, and test-retest results have not been found to be statistically different (Metman, et al., 2004).

Part III – motor examination

The motor subsection consists of 14 questions which ask the patient to perform tasks such as opening and closing their hand in rapid succession, the movement of major joints and initiation of movement such as arising from their chair. The severity of symptoms is assessed by the examiner for all questions. For some questions, such as facial expression the examiner rates the patient's severity of symptoms without the patient being required to participate. The score for each question is added and the patient is given a total between 0 – 56 where a higher score indicates more severe impairment.

Part V – Hoehn and Yahr Stage

The Hoehn and Yahr exam requires the examiner to subjectively define the patents stage of disease. Stages range from stage 0 to stage 5 where stage 0 is assigned if the patient shows no sign of disease and stage 5 if the patient is wheelchair bound or bedridden unless aided. The

examiner asks the patient to perform a series of tasks or movements, assigning them a score for each task. Tremor at rest is also examined during this procedure without the patient being required to participate. Scores are added to give an overall total for each component which translates to the Hoehn and Yahr stage.

5.3.2.2 Significant Other Interview

The significant other interview is completed with L.L. in the second session and includes: the Adaptive Behaviour Assessment System – second edition (ABAS: Harrison & Oakland, 2003); the Neuropsychiatric Inventory (Cummings, et al., 1994); the One Day Fluctuation Assessment Scale (Walker, et al., 2000) and the Activities of Daily Living – International Scale (ADL: Reisberg, et al., 2001). For each questionnaire the examiner reads out the questions to the significant other and they are asked to rate the level of patient symptoms using different Likert scales. The entire procedure is completed within an hour.

For the healthy control participants the procedure is slightly different. At the first session they are asked to take home a booklet for their significant other to complete. The booklet includes detailed instructions on how to answer each questionnaire and includes the ABAS and the ADL. The clinical dementia rating scale (CDR: Morris, 1993) is also included as a measure of cognition and includes tests of memory, awareness, judgement and problem solving abilities.

The Adaptive Behaviour Assessment System – second edition

The ABAS consists of several subscales which measure the patient's abilities across several settings. Each subscale consists of 20-27 questions which examine: levels of communication; ability to use the community; functional academics; home living; health and safety; leisure; self care; self direction and work. The significant other is required to answer each question using a Likert scale of 0 = 'is not able,' to 3 = 'always or almost always when needed.' The participant is given a total score for each subscale by adding the number given for each question. Provision is made for those questions that the significant other needs to guess or does not know the answer to.

The Neuropsychiatric Inventory

The Neuropsychiatric Inventory measures 10 behavioural disturbances that commonly occur in dementia patients including delusions, hallucinations, aggression, depression and anxiety. Within each subsection a screening question is first asked to ascertain if the behavioural

disturbance is present. This is a simple question to which the significant other either responds 'yes' or 'no' If the answer is 'no' the examiner moves to the next section. If the answer is 'yes' the significant other is asked to rate the severity and frequency of the behaviour using the below scale.

For each sub-question, the informant is asked to rate the:

- frequency: 1 = 'occasionally, less than once per week,' to 4 = 'very frequently – essentially continuously present'
- severity: 1 = 'mild, symptom is distressing but usually responds to redirection or reassurance,' to 3 =' marked – symptom is very distressing and a major source of suffering for the patient' and
- distress: 0 = 'not at all' to 5 = 'very severely or extremely.'

For each subsection the significant other is also asked to rate their own distress that is associated with the patients behaviour before moving on to subsequent sections.

The One Day Fluctuation Assessment Scale

This scale gives a measure of the patient's behaviour over the previous 24 hours and includes several subsections which measure areas of confusional behaviour (falls, fluctuations in cognitive functioning, drowsiness, attention, disorganised thinking, altered level of consciousness and communication) with specific questions such as 'has (the patient) fallen in the last 24 hours' and 'did (the patient) have difficulty focusing attention throughout the day?' The scale for subsections 1-3 begin with a dichotomous 'yes/no' question relating to the presence of the behaviour. If the answer is yes the significant other is asked to state how many times the behaviour occurred or how much of the day (25%, 25-75% or 75%+) the behaviour was present for. Items 4-5 only require the dichotomous answer and item 7 asks how well the patient understands communication using the scale of 1 – 'understands almost everything you communicate' to 3 – 'understands almost nothing of what you communicate.' The scores for each subsection are summed to provide a severity score for fluctuating confusion ranging from 0 to 21.

Activities of Daily Living – International Scale

The ADL is a 40 item questionnaire which measures the global and cognitive process of dementia. Questions include 'does the patient have difficulty putting household items in the right places?' and 'does the patient have problems remembering important dates and events?' The significant other is asked to rate the severity of difficulty using a 4 point Likert scale (0 =

'never' - 3 = 'always'). The score for each question is then added to give a total severity score out of 160.

Clinical Dementia Rating Scale

The CDR is only given to the significant other of the control participants. This rating scale consists of several subsections which address memory, orientation, judgement and problem solving, community, social and home and hobbies of the participant. Within each subsection the significant other rates the severity of symptoms using the options of: usually; sometimes; rarely or don't know. Some questions also require a brief explanation about the behaviour.

5.3.3 Measures of Global Functioning and Pre-Morbid Ability

From the initial NP1 and NP2 batteries, test scores from the following neuropsychological exams were also included in this thesis. Measures of global function were assessed by the Mini Mental State Examination (MMSE: Folstein, Folstein, & McHugh, 1975), the Montreal Cognitive Assessment (MoCA: Nasreddine, et al., 2005) and the Alzheimer's Disease Rating Scale (ADS: Rosen, Mohs, & Davis, 1984). The Wechsler Test of Adult Reading (WTAR: Wechsler, 2001) was also included to give a measure of pre morbid functioning. There were no deviations from the normal administration instructions for any of the tests.

5.3.3.1 The Mini Mental State Examination

The MMSE is a brief screening instrument for dementia, used to give an overview of global function. The test consists of 30 questions which cover a small set of verbal functions, memory and construction abilities simply and quickly. The verbal functions consist of an orientation task where the participant is asked the year, season, month, date and day and a language test where they are asked to name a series of objects, repeat a phrase and follow separate written and verbal commands. The construction task is a combination of following the verbal command to fold a paper in half and copying an interlocking pentagon design. For the attention task there are two options. In this case the total score for MMSE is based on the inclusion of the 'serial sevens' task in which the participant is asked to deduct 7 from a series of numbers, beginning at 100. This replaces the 'WORLD' task in which the participant is asked to spell 'world' backwards, as serial sevens is considered to be more challenging and is used in the diagnosis of dementia for Parkinson's disease (Dubois, et al., 2007). The outcome measure for the MMSE is the number of correct answers, where the participant is awarded one point for each answer out of a possible 30. For this study, age and education adjusted

scores were then calculated based on normative data. There is no time restriction on the MMSE but in its entirety it is expected to take between 5-10 minutes.

5.3.3.2 The Montreal Cognitive Assessment

As the MMSE is not as sensitive to the milder forms of cognitive impairment evident in Parkinson's disease and is prone to ceiling effects (Zadikoff, et al., 2007), the MoCA was administered as an additional measure of global function. The MoCA is also a brief screening tool but was developed specifically for the detection of mild cognitive impairment in patients who normally perform within the normal range on the MMSE (Nasreddine, et al., 2005). The test is the same size as the MMSE, consisting of 30 questions which cover: short term memory recall; visuospatial abilities; executive function; attention and working memory; language and orientation to time and place. The stimuli for the short term memory task are two learning trials of five nouns, and delayed recall is measured after 5 minutes. Visuospatial ability is measured from a clock-drawing task and a cube copy task. Executive function is measured using a smaller section of the Trail Making Test (TMT) - B task, a verbal fluency task and a verbal abstraction task. Attention and working memory are measured together using a test of sustained attention where the participant is required to detect a target letter from a series of stimuli letters; a serial subtraction task and the digits forward and backwards task (abridged to 5 forwards and 3 backwards stimuli). Language is measured through a naming task where participants are required to name three animals they are unlikely to be familiar with and the repetition of complex sentences. Orientation to time and place is also evaluated through questioning of the date, month, year, day, place and city. One point is awarded for each correct answer and age and education adjusted score then gained from the normative data. The MoCA is expected to take a maximum of 10 minutes.

5.3.3.3 The Wechsler Test of Adult Reading

The Wechsler Test of Adult Reading is used as a measure of premorbid function because, unlike other cognitive abilities, reading recognition is relatively stable in a diseased or ageing brain (Strauss, Sherman, & Spreen, 2006). The test stimuli are 50 words with irregular pronunciation that the participant has to read out to the examiner. Irregular pronunciation is used so that the participant cannot apply currently known pronunciation rules and has to rely solely on the word having been previously learned. The participant is awarded one point for

every correct answer which can then be converted into a WTAR standard score based on normative tables. There is no time constraint for this examination.

5.3.4 Movement Disorder Society Task Force Recommended Cognitive Domains

Following the recommendations of the Movement Disorder Society Task Force (MDS: Emre, et al., 2007), the cognitive profile of participants was examined in four key areas of function: attention; memory; executive function and visuospatial function. Several different tests were used for each cognitive domain. For each cognitive test the patients score was converted to a standard score based on age and education adjusted normative data. These standard scores were then averaged for each cognitive domain and an overall Z score created from each average domain score. The purpose, procedure and outcome measure for each test are outlined within their relevant domain below with the order of administration is given in *Table 5-1*.

5.3.4.1 Attention/working memory/processing speed

Attention/working memory/processing speed was primarily examined using components of the Weschler Adult Intelligence Scale (WAIS-IV: Wechsler, 2001), the Delis-Kaplan stroop task (Delis, Kaplan, & Krammer, 2001) and the trail making task from the Army Individual Test battery (*Army Individual Test Battery: Manual of directions and scoring*, 1944).

Digit Span Forwards and Backwards

Digit span modality tests are used as a measure of the working memory component of verbal IQ (Strauss, et al., 2006). Both the digit span forwards and digit span backwards tasks are recommended by the MDS to measure short term and working memory (Dubois, et al., 2007). The digit span test has been shown to be sensitive to the development of PD dementia (Woods & Troster, 2003a). This sample consisted of patients who initially presented as non-demented but who were diagnosed with PD-D at one year follow up. The baseline scores on the digits backwards subset exhibited diagnostic accuracy in predicting PD-D at follow up. The stimuli for the digits tests are verbal, a series of non sequential numbers are read to the participant at a rate of one per second. Each subset is presented twice using different digits. Part 1 measures forwards digit span using stimuli subsets of 2-9 numbers and Part 2 measures backwards digit span using stimuli subsets of 2-8 numbers. Once the entire sequence has been presented the participant is instructed to repeat the sequence of digits back to the examiner in the order they were given (Digit span forwards) or in reverse order (Digit span backwards) without any

constraint on time. Scoring is contingent on the participant correctly repeating all numbers in the trial for which they gain one point.

Digit Ordering Test

The digit ordering test is sensitive to working memory deficits in PD without dementia (Hoppe, Muller, Werheid, Thone, & von Cramon, 2000) and is recommended by the MDS to measure working memory deficits in PD (Dubois, et al., 2007). Similar to the digit span modality tests, the digit ordering test consists of stimuli subsets of 3-8 numbers which are presented to the participant in two trials of independent stimuli. After presentation of the digits the participant is required to repeat the numbers back to the examiner in ascending numerical order, gaining one point for the completion of each trial.

Map Search

The map search task is a test of everyday memory and attention (Robertson, Ward, Ridgeway, & Nimmo-Smith, 1996) that requires the participant to ignore all distracter stimuli and identify the target stimuli – a measure of focussed attention. The map search has been applied in one of our own PD samples (Dalrymple-Alford, et al., 2011) as part of the proposed diagnostic criteria for PD-MCI and shows adequate discrimination between PD-MCI and PD-N and between PD-D and PD-MCI. The map search also has a 63-72% sensitivity in discriminating between MCI and dementia of the Alzheimer's type (De Jager, Hogervorst, Combrinck, & Budge, 2003). The apparatus in this case is a large laminated street map and the target stimuli are restaurant symbols. The participant is required to use a whiteboard marker to circle all the target stimuli. This task is time constrained to two minutes. The outcome measure is the number of correct (hits) and incorrect responses which are recorded separately in two, one minute intervals. The total number of hits and incorrect responses is also calculated as an outcome measure.

Stroop Task

The Stroop task measures selective attention failures and is recommended by the MDS in the diagnosis of PD dementia (Dubois, et al., 2007). The Stroop consists of three main trials: the colour trial, word trial and the stroop task proper. The colour trial requires the participants to merely interpret patches of block colour and state the name of it. Similarly, the word trial requires them only to read a printed word. Participants are first required to complete the colour trial and the word trial to ensure competency for the completion of the Stroop task

proper. The stoop task proper is hampered by a combination of the previous trials and incongruent conditions whereby the printed word of the colour is written in ink of a different colour such as the word 'RED' written in green ink. All three trials have a good discrimination between PD-N and PD-MCI (AUC = 68-81) and between PD-MCI and PD-D (AUC = 83-85) when examined in one of our own PD samples (Dalrymple-Alford, et al., 2011).

Colour Trial

The Stroop colour trial measures the participants visual ability. The stimuli for this trial is several rows of colour patches. Colours are restricted to primary colours (red, blue, yellow and green). Operating in left to right, top to bottom sequential order the participant is required to name the colour patches. The outcome measure is the number of correct responses with one point awarded for each correct response. This trial is limited to 90 seconds.

Word Reading Trial

The Stroop word reading trial measures the participants reading competency which is essential for the completion of the Stroop task proper. For this trial the stimuli is several rows of colour words 'RED, GREEN, BLUE' printed in black ink. Similar to the colour trial the participant is required to read each of the words and given one point for each correct response. This trial is also limited to a total of 90 seconds.

Trail Making Test – Part A

The trail making test is a measure of scanning and visuomotor tracking and is sensitive to cognitive changes in mid stage Parkinson's disease patients (Hietanen & Teravainen, 1986). The TMT is divided into two parts and it is one of the tests recommended by the MDS to measure abilities of set activation, set shifting and set maintenance (Dubois, et al., 2007). The stimuli for both parts is the same, the participants are presented with an A4 sheet of paper on which is printed a series of randomly positioned sequential numbers (1-25), each encased in small circles. Part A requires participants to link the numbers together in numerical ascending order using a pencil. Participants are instructed to complete the task 'as fast as you can' with the outcome measure the total time to completion.

5.3.4.2 Executive function

The test that is primarily used for executive function measures is the Delis-Kaplin Executive Function System (Delis, et al., 2001) which is nine independent tests that are designed to measure the fluency and cognitive flexibility aspects of executive function. The following subsets of this examination were administered independently in this study, only the category fluency and category switching test were given consecutively.

Letter Fluency (Version A)

It is recommended that verbal fluency tests are used in the criteria to diagnose PD dementia (Dubois, et al., 2007) as they are a relatively pure measure of executive function and performance is significantly reduced in patients with Parkinson's disease dementia (Pillon, Dubois, & Agid, 1991). Together with verbal memory measures, verbal fluency performance is also reduced in those PD patients with cognitive impairment without dementia (Jacobs, et al., 1995; Levy, et al., 2002). The letter fluency task consists of three trials and requires the participant to draw on skills of processing speed, task initiation and simultaneous processing abilities. The participant is required to list as many words as they can think of beginning with the letters 'F,' 'A,' and 'S.' Words are not allowed to be proper nouns and each trial is restricted to a maximum of 60 seconds. The outcome measure of interest is the number of correct words that are able to be generated.

Action Fluency

Not specifically recommended by the MDS (Dubois, et al., 2007), action fluency is nevertheless sensitive to the cognitive changes between PD-N and PD-MCI and between PD-MCI and PD-D in our own sample (Dalrymple-Alford, et al., 2011). Similar to the letter fluency task, the participant is required to list as many verbs (explained as 'things that people do') as they can in a 60 second time frame. The outcome measure is the number of correct words that are able to be generated.

Category Fluency

Category fluency is a verbal fluency task that is recommended to aid in the diagnosis of dementia (Dubois, et al., 2007). Category fluency uses the same processing and retrieval skills as the letter fluency task but requires that the participant follow the additional rule of semantic category. The D-KEFS categories stipulates that the participant must list as many animals as they can think of in Trial 1, followed immediately by as many boys names in Trial

2, requiring the participant to employ a selection and discounting process for each generated word. Each trial is restricted to a time of 60 seconds and the outcome measure is the number of correctly generated words.

Category Switching

The category switching task occurs immediately subsequent to the category fluency trails and requires participants to rapidly generate both fruits and furniture (switching between categories for each word). Significantly impaired in both PD-D and PD-MCI (Dalrymple-Alford, et al., 2011), category switching is considered a measure of cognitive flexibility. This test consists of one trial lasting 60 seconds where the outcome measure is the number of correct words in the category switches.

Trail Making Test – Part B

Adding to the requirements of Part A of the TMT, Part B is a measure of divided attention and cognitive flexibility. The stimuli for this test are similar but the numbered circles from the original test are interspersed with circled letters. Participants are required to connect sequential numbers and letters, alternating between each (for example, 1-A, 2-B, 3-C). The outcome measure is total time to completion.

Stroop Total

The total Stroop task measures selective attention. The stimuli in this task are conflicting and presented in either a congruent or incongruent manner. In the congruent set, the word is written in the colour it stands for (i.e.: the word 'RED' is written in red ink), in the incongruent set, the word is written in an opposing colour (i.e.: the word 'RED' is written in green ink). For both sets the participant is required to name the colour the word is printed in and ignore what the word says. Findings suggest that patients who struggle on the incongruent trials have difficulty concentrating and ignoring distractions (Strauss, et al., 2006). The outcome measure is the total score where one point is given for each correct response. This trial is time constrained to 180 seconds.

5.3.4.3 Visuoperceptual/Visuospatial function

This domain encompasses measures of spatial awareness and stimuli perception. It is difficult to gain a 'pure' measure of spatial awareness or stimuli perception as there is a great degree of overlap between the two functions. The Judgement of Line test (JOL: Benton, Varney, &

Hamsher, 1978) mainly requires spatial awareness while the fragmented letters test from the Visual Object and Space Perception Battery (VOSP: Warrington & James, 1991) mainly measures stimuli perception. The copy subset of the Rey Complex Figure Test (Meyers & Meyers, 1995) encompasses both spatial awareness and perception skills.

Judgement of Line Orientation

The JOL was applied as a measure of visuospatial ability. This test is sensitive to pure visuospatial deficits in Parkinson's disease as the high failure rate on this measure is independent of cognitive ability, disease severity or PD duration (Montse, Pere, Carme, Francesc, & Eduardo, 2001). The JOL stimuli can be any two lines which vary in angle from 0 – 180 degrees and a separate picture where all possible line angles are presented together. There are 11 possible angle options. The participant is presented with the line pairs and asked to match them to the test picture. There are 30 test stimuli which are presented and the participant gains one point only if they correctly identify the angle of both lines in the pair.

Fragmented letters

The fragmented letters subset of the VOSP is a measure of visual perception and is one of the tests recommended by the MDS in the diagnosis of dementia (Dubois, et al., 2007). The participant is presented with 20 large alphabet cards that have each been altered until only 30% of the original shape remains. The participant gains one point for each correctly identified shape and is given up to 10 seconds per card before being told to 'guess if you are not sure.' The outcome measure is the number of correctly identified letters.

Rey Complex Figure – Copy Trial

The RCF stimulus is a complicated diagram comprised of several different shapes and lines of varying angle and size. The figure is placed in front of the participant in a horizontal direction and they are required to copy the figure exactly as they see it without moving or rotating the test stimulus. The copy trial measures both visual perception, when the participant is required to interpret the stimulus; and perceptual organisation/spatial abilities when the participant is required to re-draw the shape. To score the participants performance on this task the examiner copies what the participant draws as they draw it, changing the colour of the pen used in order to note the sequence in which the figure is completed. For this study the pen colour was changed at an interval of 60 seconds. The participant is given an unlimited amount of time to copy the diagram but time to completion is recorded. The

scoring system is based on the participants' placement of the stimulus, the size of the components and accuracy of the drawing. The figure is divided into 18 scorable units which are individual areas or details of the figure. A correctly placed and proportional copy of each unit earns 2 points so the maximum score for the total figure is 36 points.

5.3.4.4 Learning and Memory

Learning and memory was measured using both verbal and visual stimuli as both functions are impaired in early PD and have been shown to be independent of medication effects (Singh & Behari, 2006). Verbal memory was examined using the California Verbal Learning Test (CVLT: Delis, Kramer, Kaplan, & Ober, 1987) which has been shown to be sensitive to memory dysfunction in early PD (Taylor, Saint-Cyr, & Lang, 1990). Visual memory was examined using the Rey Complex Figure Test (RCFT: Meyers & Meyers, 1995) which is sensitive to cognitive dysfunction in PD without dementia (Uc, et al., 2005). Although recognition memory appears to be intact in early PD, evidence is starting to suggest this is not the case in PD-D (Whittington, Podd, & Kan, 2000). The aim of this study was to measure cognitive dysfunction in the early and mild cognitive impairment stages of PD as well as in the PD-D stage. Therefore, in accordance with recommendations made by the movement disorder society task force (Emre, et al., 2007), the recognition trials of the CVLT and RCFT were not included in this thesis, although they were administered for the wider PD and biomarker studies.

<u>California Verbal Learning Test – Acquisition trials</u>

The CVLT is designed to examine the use of semantic association as a strategy for word learning. The stimuli for each CVLT list is 16 words which each belong to one of four categories. The lists used for this study were List A, which categorised the words into vegetables, animals, ways of travelling and furniture and List B, which, in addition to the vegetables and animals category from List A also categorised words into musical instruments and parts of furniture. For the acquisition trial the entire list is read to the participant at a slow, even pace so that the list takes 12-14 seconds to read. The participant is given four trials to list all the words they remember, with the list read to them again at the beginning of each trial. The total number of correct words are recorded along with the total number of repetitions and total number of words that were not on the list but were given as being remembered from the list (intrusions).

California Verbal Learning Test – short delay (30sec) free recall trial

The short delay free recall trial occurs after a 30 second distractor task. In this case the distractor task required the participant to count backwards from 100. The participant has not previously been forewarned that they will be required to remember the CVLT word list but is then required to list as many of the words as they can remember. Scoring is conducted in the same way as for the acquisition trials.

California Verbal Learning Test – long delay (10 min) free recall trial

The long delay free recall trial occurs after a delay of 10 minutes from the short delay free recall trial. Again, the participant has not been told that they will be tested on the words a second time. The participant is given one trial to recall as many words as possible, with scoring occurring in the same manner as in previous trials.

Rey Complex Figure Test – short delay (3 min) free recall trial

The short delay free recall trial of the RCFT was administered after an interval of approximately 3 minutes. The participant had not been previously warned that they would be recreating the figure from memory. The participant was given a sheet of paper and instructed to draw the complex figure from memory with scoring occurring in the same manner as for the copy trial.

Rey Complex Figure Test – long delay free recall trial

The long delay free recall trial is administered 30 minutes after the stimulus is first presented in the copy trial, considered to be a length of time that is sufficient for retention to have occurred. The procedure and scoring did not vary from that which was conducted in the previous trials.

Table 5-1: Study procedure

Recruitment procedure for the ongoing work at NZBRI

Patients

- Identified from specialist clinic attendance
- Recruitment begins by mail contact
- Patient expresses interest
- Patient is contacted by phone and appointments are arranged in a way that ensures they are all completed within a month

Healthy control subjects

- Recruited through community advertising
- Added to the database held at the NZBRI research clinics
- Control subjects are only contacted if they meet the eligibility criteria for a specific study

MRI procedure

The participant undergoes a complex scanning procedure where T1, T2, and DT images are obtained.

Significant other interview

Patients

The patient is required to bring a significant other to the second session. This person will then complete the interview in a separate room to the patient.

Healthy control subjects

The significant other of the healthy control participants is posted the questionnaire and is able to complete it at home prior to the first session.

Neuropsychological testing procedure

Session 1

1. Consent form
2. Health checklist and background for PD
3. MMSE
4. GDS
5. WTAR
6. CVLT-II Short Form – free recall trial
7. VOSP – shape detection and incomplete letters
8. Digit span – forwards
9. Digit span – backwards
10. Digit ordering
11. CVLT-II Short Form – long delay free recall
12. Letter fluency
13. JOL Orientation – Form H
14. Category Fluency and Category Switching
15. Trail Making – Part A
16. Trail Making – Part B
17. WASI – Matrix Reasoning
18. Boston Naming Test

Session 2

1. Parkinson's disease questionnaire
2. RCFT – copy trial
3. RCFT – immediate recall trial
4. MoCA
5. RCFT – delayed recall trial – short delay
6. Stroop task – colour trial
7. Stroop task – word trial
8. Stroop task – test trial
9. RCFT – delayed recall trial – long delay
10. RCFT – recognition trial
11. DRS-2
12. TEA map search
13. ADAS-cog
14. SCOPA-Cog delayed recall
15. ADAS-cog continued

Recruitment procedure for this thesis

Patients

1. Data from all patients that had completed NP testing by 25 March 2010 was collected
2. Images for each patient were collected. Those patients for who the scanning procedure had not been completed by 13 March 2010 were discounted.
3. Exclusion criteria specific to this thesis was applied to all remaining participants.

Healthy control subjects

1. Data from all healthy control subjects was collected from the database.

2. Images for each participant were collected, those subjects for whom imaging had not yet been completed were discounted

3. Exclusion criteria for this thesis was applied to all remaining participants

MMSE: Mini mental state examination; **GDS:** geriatric depression scale; **WTAR:** Weschler test of adult reading; **CVLT:** California verbal learning test; **VOSP:** the visual object and space perception battery; **JOL:** judgement of line orientation; **WASI:** Wechsler abbreviated scale of intelligence; **RCFT:** Rey complex figure test; **MoCA:** Montreal cognitive assessment; **DRS:** dementia rating scale; **TEA:** test of everyday attention; **SCOPA:** Scales for outcomes in Parkinson disease; **ADAS:** Alzheimer's disease assessment scale

5.1 Clinical diagnosis of Parkinson's disease

Diagnosis of Parkinson's disease was made following the UK Parkinson's disease brain bank criteria (Hughes, et al., 1992) by a qualified neurologist (T.A) prior to this study being conducted. This criteria (*Figure 5-2*) outlines three steps in the diagnosis of Parkinson's disease: the diagnosis of Parkinsonian syndrome; any criteria which would exclude Parkinson's disease cannot be met and there must be supportive positive criteria for Parkinson's disease.

UK Parkinson's Disease Society Brain Bank clinical diagnostic criteria

Step 1 Diagnosis of Parkinsonian syndrome

- Bradykinesia (slowness of initiation of voluntary movement with progressive reduction in speed and amplitude of repetitive actions)
- And at least one of the following:
    - muscular rigidity
    - 4-6 Hz rest tremor
    - postural instability not caused by primary visual, vestibular, cerebellar, or
    - proprioceptive dysfunction.

Step 2 Exclusion criteria for Parkinson's disease

- History of repeated strokes with stepwise progression of parkinsonian features
- History of repeated head injury
- History of definite encephalitis
- Oculogyric crises
- Neuroleptic treatment at onset of symptoms
- More than one affected relative
- Sustained remission
- Strictly unilateral features after 3 years
- Supranuclear gaze palsy
- Cerebellar signs
- Early severe autonomic involvement
- Early severe dementia with disturbances of memory, language, and praxis
- Babinski sign
- Presence of cerebral tumor or communicating hydrocephalus on CT scan
- Negative response to large doses of levodopa (if malabsorption excluded)
- MPTP exposure

Step 3 Supportive prospective positive criteria for Parkinson's disease
(Three or more required for diagnosis of definite Parkinson's disease)

- Unilateral onset
- Rest tremor present
- Progressive disorder
- Persistent asymmetry affecting side of onset most
- Excellent response (70-100%) to levodopa
- Severe levodopa-induced chorea
- Levodopa response for 5 years or more
- Clinical course of 10 years or more

Figure 5-2: Parkinson's disease criteria
UK Parkinson's Disease Society Brain Bank clinical diagnostic criteria (Hughes, et al., 1992) used for the diagnosis of PD.

5.2 Diagnosis of Cognitive Impairment

5.2.1 Parkinson's disease with dementia

Parkinson's disease dementia was diagnosed based on the Movement Disorders Task Force criteria (Dubois, et al., 2007; Emre, et al., 2007). The criteria for a diagnosis of probable PD-D stipulates that the patient must have: 1) a diagnosis of PD – made according to the Queen Square Brain Bank criteria; and 2) a dementia syndrome of insidious onset and slow progression that has developed within the context of established PD. The patient must also show at least three associated clinical features, two of which are cognitive and one of which is behavioural. Cognitive features include impairment in the MDS recommended cognitive domains of attention, executive functions, visuo-spatial function, memory and language. Behavioural features include apathy, personality changes, hallucinations, delusions and excessive daytime sleepiness. Features such as the co-existence of any other abnormality which may cause cognitive impairment (vascular disease or an unknown time between the development of motor and cognitive symptoms) make the PD-D diagnosis uncertain and cannot be present. Features that suggest another condition also may not be present and include: cognitive and behavioural symptoms appearing only within other conditions such as a systemic disease or drug intoxication; major depression according to DSM-IV criteria; or any features that are compatible with probable vascular dementia criteria. Following the criteria of Emre, et al., (2007) the patients' symptoms must be severe enough to impact on their activities of daily living and cannot be attributed to their motor impairment.

5.2.2 Mild cognitive impairment

A diagnosis of mild cognitive impairment was made according to New Zealand Brain Research Institute criteria. This criteria has been examined relative to other methods of MCI diagnosis and found to be a valid and reliable measure of cognitive impairment which optimises the trade off between identifying cases with poor cognition and avoiding those with relatively good cognition (Dalrymple-Alford, et al., 2011). Aspects of this criteria, namely those requiring that impairment is present in at least one test in each of two or more separate cognitive domains have also recently been included in MDS task force guidelines for diagnostic criteria for mild cognitive impairment (Litvan, et al., 2012). The full criteria requires that participants have unimpaired activities of daily living (verified in a structured interview with the patients' significant other) but exhibit cognitive decline,

defined as test scores of at least 1.5 standard deviations below that of normative data on at least two measures within the four MDS cognitive domains (executive function, working memory, attention and visuospatial functions). This criteria was used to identify MCI in both the healthy control group and the Parkinson's disease group for both the PD and biomarkers studies.

5.2.3 Parkinson's disease with no cognitive impairment

Parkinson's disease patients were considered to be unimpaired if they did not meet the criteria for MCI.

5.3 MR and DT Imaging

During the scanning procedure several scanning sequences were utilised in order to maximise the time and staff resources that were allocated to the wider study. Consequently, although the sequences required for this study were short, the total scanning time was around an hour for each participant. In total, those sequences which gave rise to a structural T1, clinical T2, T2 flair and T2*, a 4D brain, arterial spin labelling (ASL) and single voxel spectroscopy in the posterior cingulate and striatum were applied. For the purpose of this study only those files needed to form the structural T1, T2 and the diffusion 4D, FA and MD images were imported for analysis.

5.3.1 The MRI machine

More advanced and less dangerous than X ray, the MRI machine enables visualisation of soft tissues without exposing the patient to harmful rays (Pooley, 2005). The New Zealand Brain Research Institute was the first place in New Zealand to install a General Electric 3 Tesla MRI machine (*Figure 5-3a*). The 3T scanner enables scans to be performed faster, at a higher resolution and with better contrast than the previously available 1.5T scanners and is especially valuable for research. The MRI scanner itself is large structure which essentially enables the patient to be slid into a strong magnetic field and operates according to the properties of magnetisation and the principals of diffusion.

A human cell is made up of 60-90% water. The properties of diffusion stipulate that this water moves slowest through barriers such as cell membranes and faster when movement is unrestricted. In the brain for example, the bodies of the neurons are more densely packed than the axons so water distributes more freely around the axons. Due to the inherently magnetic properties of the water molecule, the MRI machine is able to

detect the speed and direction the water molecules move in. This in turn gives rise to the MR signal which is then able to be detected in the MRI machine. The magnetic field within the MRI machine is able to be systematically altered which allows for visualisation of water movement in multiple directions and areas of the brain.

The strength of the magnetic field depends on the number and strength of each type of MRI magnet. The MRI scanner has three main magnet types. The permanent magnet is the largest and is always charged – requiring no electricity to generate the magnetic field. Gradient magnets are wound around a cylinder within the scanner and only create a magnetic field when electricity runs through them. By selecting a gradient magnet (denoted Gx, Gy and Gz) for the current to run through, the MR signal for a certain direction can be mapped. The gradient magnets operate in three main directions. The X gradient magnet allows the scanner to image the brain in coronal sections, the Y gradient in sagittal sections and the Z gradient in axial or horizontal sections (Pooley, 2005).

5.3.2 Image acquisition

All imaging was performed using the General Electric (GE) 3 Tesla HDx MRI scanner using an eight channel head coil. Structural MR images were acquired with a T1 weighted, three dimensional spoiled gradient recalled (SPGR) echo acquisition (TE/TR = 2.8/6.6ms, TI = 400ms, flip angle = 15 deg, acquisition matrix = 256 x 256 x 170, slice thickness = 1mm (no gaps), FOV = 250 x 250mm^2 reconstruction matrix = 512 x 512 x 170, reconstructed voxel size 0.5 x 0.5 x 1 mm^3), scan time = 5min 6sec.

Diffusion weighted images were acquired with a 2D diffusion-weighted, spin echo, echo planar imaging sequence, with diffusion weighting in 28 uniformly distributed directions (b = 1000s/mm^2) and 4 acquisitions without diffusion weighting (b = 0s/mm^2): TE/TR = 86.4/13000 ms, flip angle = 90 deg, acquisition matrix = 128 x 128 x 48, slice thickness = 3mm (no gaps), (FOV = 240mm), voxel size = 1.88x1.88x3mm^3 scan time = 7min 9sec.

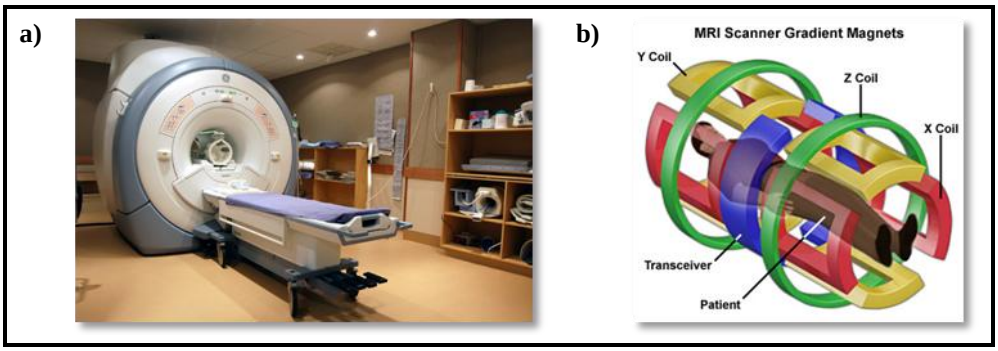


Figure 5-3: The GE 3T MRI machine
a) The 3T MRI machine housed at the New Zealand Brain Research Institute, **b)** the configuration of the gradient magnets within the MRI machine that determine the direction that the MR signal is mapped in. Image b from Coyne (2012).

5.3.3 Image creation

An MR image is essentially a snapshot of the distribution of the MR signal (Buxton, 2002). Individual spatial locations within the patient are able to be localised by combining radiofrequency pulses - which serve to excite the water molecules, and magnetic field gradients which orientate and move the water molecules in the desired direction (Busherg, Seibert, Leidholdt, & Boone, 2002). A temporary matrix comprising several 3D cubes (voxels) is created and each cube within the matrix is able to be filled with the MR signal by altering the gradient of the magnetic field and filling in every row and column for each brain slice. Once in the matrix, mathematical transformations are applied to each row and column of voxels. This results in an image where the horizontal and vertical axis correspond to the horizontal and vertical spatial frequencies of the original matrix (Hoa, 2009).

5.3.3.1 The MR Signal

The MR signal is created when the water molecules within the human body move in response to being placed in the main magnetic field of the MRI machine. Two hydrogen atoms and one oxygen atom form to make a water molecule (H_2O). The protons of the hydrogen atom are inherently positively charged (magnetic moment) and can be detected by the MRI's receivers. When first placed in an MRI scanner the proton orientates to the direction of the main magnetic field (B_0), *Figure 5-4*. The movement of the proton creates an MR signal, the strength of which varies according to the density of the protons and the time it takes for magnetised protons to return to their normal state parallel or perpendicular to the main magnetic field.

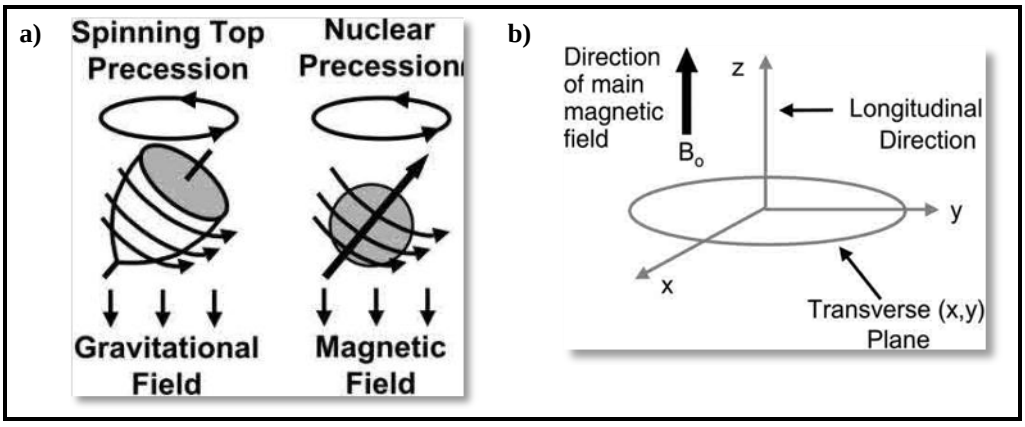


Figure 5-4: The creation of the MR signal
a) The precession of the proton atom within the water molecule that allows for the creation of the MR signal. Image from Mori & Zhang, (2006), **b)** schematic diagram of the direction of the magnetic field. Image from Pooley (2005).

The main magnetic field is always in the Z direction and proton movement is around this axis. The force of the magnetic field causes the proton to 'precess,' that is to wobble about its' axis whilst trying to maintain the original orientation. The precession of the protons occurs at a speed proportional to the strength of the magnetic field (Larmor frequency) and results in a voltage which can be read in the receiver coil of the machine. The voltage shows the direction the proton is orientated to. In the absence of the magnetic field, the spin of the protons will naturally relax and settle back into a stable state over time. The time taken to return to stable state varies and is affected by processes that reduce magnetisation. It is the measurement of this loss of signal that gives rise to the brain images. The image is created when energy is first introduced into the stable state by applying an electromagnetic wave at the same frequency as the Larmor frequency. This radiofrequency energy (RF) is generated by rapidly changing magnetic and electric fields within the scanner. The RF energy is transmitted for a short period of time (RF pulse) and excites the spin system of the protons from the stable state. The effect of this electromagnetic wave is to induce an alternating voltage of the same frequency as the Larmor frequency in the receiver coil which is interpreted as the MR signal.

This signal fades due to one of two processes which reduce magnetisation in the transverse plane. Spin-lattice interaction (T1 Relaxation) is the primary source of contrast in the T1 images while spin-spin interaction (T2 Relaxation) is the source of contrast in the T2 images.

5.3.4 Structural T1 Image: Longitudinal relaxation

The longitudinal magnetization (Z) (*Figure 5-4*) can be rotated exactly into the transverse plane (XY) by a radiofrequency pulse which is strong enough and long enough to tip the magnetisation exactly 90^0 (RF 90^0). After a RF 90^0 pulse the protons are aligned with the transverse plane and the longitudinal plane is zero. As the transverse magnetization (Mxy) decays, the magnetic moments of the protons realign with the Z axis of the magnetic field (longitudinal relaxation). The relaxation rate varies for protons associated with different tissues.

For the T1 image, relaxation occurs through the transfer of energy from the protons to the lattice field. The lattice field is the complex magnetic field that groups of nuclei create to distribute energy. The lattice field is created when a nucleus in a low energy state interacts with a nucleus in a high energy state. This contact causes the energy to be evenly distributed between the two nuclei. When the radiofrequency pulse is applied, more energy is gained by the nuclei and again distributed throughout the lattice. The spin-lattice relaxation refers to the time it takes for the spins to give the energy obtained by the RF pulse back to the surrounding lattice. The transfer of energy restores the protons to their equilibrium state (Mori & Zhang, 2006). As different tissues have inherently different T1 values, this is one basis of introducing tissue contrast into the MR image. Myelin causes white matter to have a shorter T1 than grey matter (Gowland & Stevenson, 2003) for example, so the T1 tissue relaxation time for magnetic strength of 1.5T of cerebral grey matter is 900ms while the tissue relaxation time for cerebral white matter is 780ms (Webb, 2003).

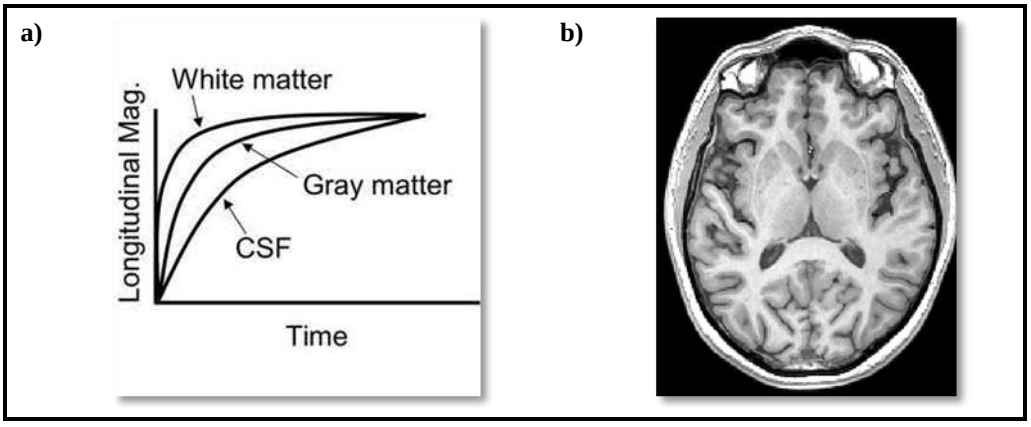


Figure 5-5: The T1 image
a) The loss of magnetisation over time in the longitudinal direction (Pooley, 2005), **b)** Horizontal section of whole brain, the T1 image provides good contrast between grey and white matter but a high level of detail is not visible. The T1 image is created using a scanning sequence with a short echo time (TE) and short relaxation time (TR) and is governed by the spin-lattice relaxation time.

5.3.5 Structural T2 Image: Transverse Relaxation

The decay of transverse magnetisation occurs as the spinning protons transfer their energy to each other, instead of to a surrounding lattice field. The spins are associated with small magnetic fields that randomly interact with each other. The movement of protons within an electromagnetic field can be described by their phase. The phase of the proton is its position on the precessional path which is expressed as an angle. For example, if proton A and B were precessing at the same speed but B was ahead of A by 10^0 then B is said to have a phase of 10^0 relative to A. After excitation, the spins precess together (phase = 0^0). This is referred to as phase coherence. All protons still rotate about the Z axis but phases differ by angles. Phase coherence is gradually lost, some spins advance while others fall behind the precessional path. When this happens, individual magnetisation vectors begin to cancel each other out instead of adding together. The vector sum of the transverse magnetization becomes smaller and smaller and finally disappears which results in disappearance of the MR signal (Weishaupt, Kochli, & Marincek, 2008). When the transverse magnetisation is completely in phase, the measured MR signal is at a maximum. When this magnetisation begins to diphase, the measured MR signal begins to decrease until the magnetisation is completely dephased and the MR signal is zero (Pooley, 2005).

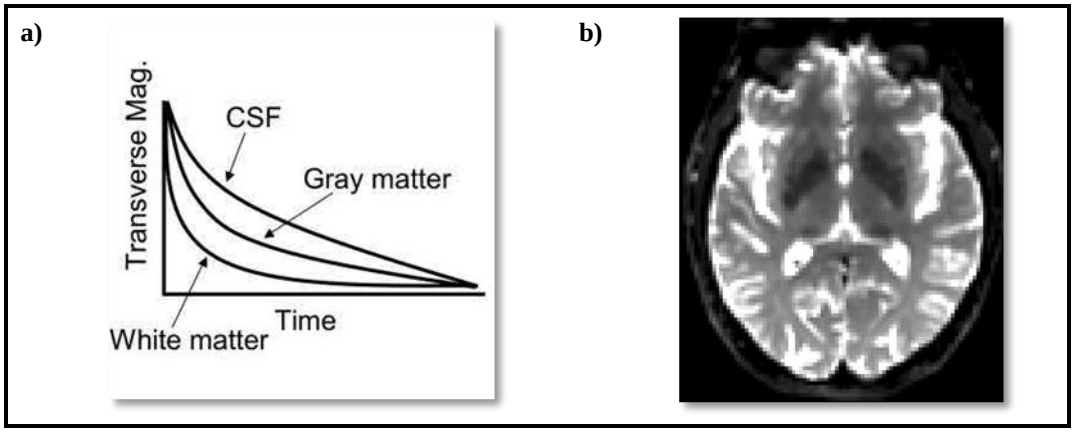


Figure 5-6: The T2 image
a) The loss of magnetisation over time in the transverse plane (Pooley, 2005), **b)** T2 horizontal section, the T2 image is governed by a spin-spin relaxation time and has a long echo time (TE) and long relaxation time (TR), where TE is the time from the application of the radiofrequency pulse to the peak of the signal induced in the coil and TR is the time from the application of one RF pulse to another RF pulse.

The T1 and T2 images are independent of each other but occur at the same time. The decrease in MR signal due to T2 relaxation occurs within the first 100-300ms of the scanning sequence and finishes before there has been complete recovery of longitudinal relaxation due to T1 relaxation (0.5 – 5 sec) (Weishaupt, et al., 2008).

5.3.6 Diffusion tensor images

The same properties relating to the detection of proton movement apply in the diffusion weighted (DW) sequences, except in this sequence there is an additional encoding process where a short, high-amplitude gradient pulse is applied that excites the spin population between the initial 90° RF and the 180° refocusing RF pulses. This additional gradient pulse accelerates the precession of some of the proton spins (dependent on their spatial location). If the gradient is applied in the Z direction for example, those protons with high Z coordinates will precces faster than those with low Z coordinates. Spins are therefore precessing (and giving rise to the MR signal) at different rates. The second step is to apply a gradient pulse of equal amplitude and duration as the first one. This causes those spins with high Z coordinates to again precess faster but since they are now orientated to the opposite direction, the spins will rephase. If the individual spins remain at exactly the same spatial coordinates during both gradient pulses the rephasing will be exact and there will be no loss of signal in the spin echo. If the protons become displaced between the pulses however then rephasing will be incomplete and there will be a loss of signal in the spin echo.

Gradients additional to the traditional X, Y and Z direction are added during the DW sequence. To measure diffusion in multiple different directions, the axes on which the diffusion weighting gradient is applied are varied, changing the motion of the water molecules. Computerised mathematics is then applied to calculate the diffusion tensor at every voxel within the image, taking into account the strength of the signal and the noise in each measurement. The combination of the increased signal and additional directions allows for an image much more sensitive to changes at the micro level than the T1 and T2 images.

The additional directions results in the creation of 1536 files, compared to the normal 170 files that are created during the T1 or T2 sequences. The DW files allow for visualisation of 48 brain slices in 32 different directions. Each direction is then combined across the total brain, forming 32 directional volume files. From here, the files are used to create one 4D image which contains the normal 3D image (X, Y, Z direction) and the level of diffusion weighting (b = 1000 or b = 0) for each direction.

The following preprocessing steps were completed in FSL, a comprehensive library of analysis tools used for MRI and DTI data created by the FMRIB analysis group (Smith, et al., 2004). The 4D image undergoes an eddy current correction to correct for movement

artifacts and is fitted to the diffusion tensor using the DTI fit function in FSL. The fit of the diffusion tensor model to the data allows for interpretation of the data in a way that incorporates all the directional information. The properties of diffusion (speed and degree of proton movement) for each voxel are then calculated and transformed mathematically to form the fractional anisotropy and mean diffusivity maps. These diffusion maps allow for cellular structure and density to be inferred from the image. The diffusion images used in this study are complied of 1.88 x 1.88 x 3 mm^3 sized voxels. Within this area, multiple bundles of axons, myelin sheaths, astrocytes and extracellular spaces will be present (Mori & Zhang, 2006) and the density of these structures is reflected in diffusion parameters.

The FA map is one of the most widely used measures of diffusion anisotropy and shows the principal direction of diffusion within a voxel. Each voxel on this map gives a numerical value representing the proportion of the diffusion tensor (D) which is due to diffusion in one direction (Wheeler-Kingshott, Barker, Steens, & van Buchem, 2003). The direction of diffusion is scaled from 0 (isotropic) to 1 (anisotropic) where isotropic diffusion indicates that proton movement is unrestricted and anisotropic diffusion means that proton movement is occurring only along one axis and is fully restricted in all other directions (Mori & Zhang, 2006). The FA of each voxel is calculated by taking the average of the diffusion tensor for that voxel using the Eig X, Y and Z files which correspond to the direction of diffusion along the lengths of the longest, middle and shortest axis of the ellipsoid (eigenvalues λ_1, λ_2 and λ_3) respectively. Eig X is the right – left direction, Eig Y is the anterior-posterior direction and Eig Z is the superior-inferior direction.

$$FA = \sqrt{\frac{1}{2}} \frac{\sqrt{(\lambda 1 - \lambda 2)^2 + (\lambda 2 - \lambda 3)^2 + (\lambda 3 - \lambda 1)^2}}{\sqrt{\lambda 1^2 + \lambda 2^2 + \lambda 3^2}}$$

where $\lambda 1$ = Eig X, $\lambda 2$ = Eig Y and $\lambda 3$ = Eig Z.

The FA map is easy to interpret as there is a great contrast between grey matter and white matter (*Figure 5-7*), which makes it ideal for defining regions of interest. The Eig X,Y and Z files can also be colour coded to the direction of the main eigenvector so that fibers orientated to the X direction are coded as red, fibers in the Y direction are coded as green and fibers in the Z direction are coded as blue. This step was completed in MATLAB version 7 ("MATLAB," 2008) which resulted in the creation of three new files (RED, GREEN, BLUE) which were then able to be overlayed onto any other image to help with defining the boundaries of the region of interest.

Interpretation of the FA map allows for inferences about the directionality of fibre tracts and the integrity of surrounding cellular structures. It is assumed that an FA value close to 1 represents diffusion in an almost uniform direction – a phenomenon typical to axonal fibre tracts. FA reduction in neurodegenerative disease has been attributed to a number of factors including demyelination, gliosis and inflammation (Assaf & Pasternak, 2008). The relationship between FA and cell density still remains controversial however, with reports of FA both increasing (Kinoshita, et al., 2008) and decreasing in densely packed areas (Stadlbauer, et al., 2006).

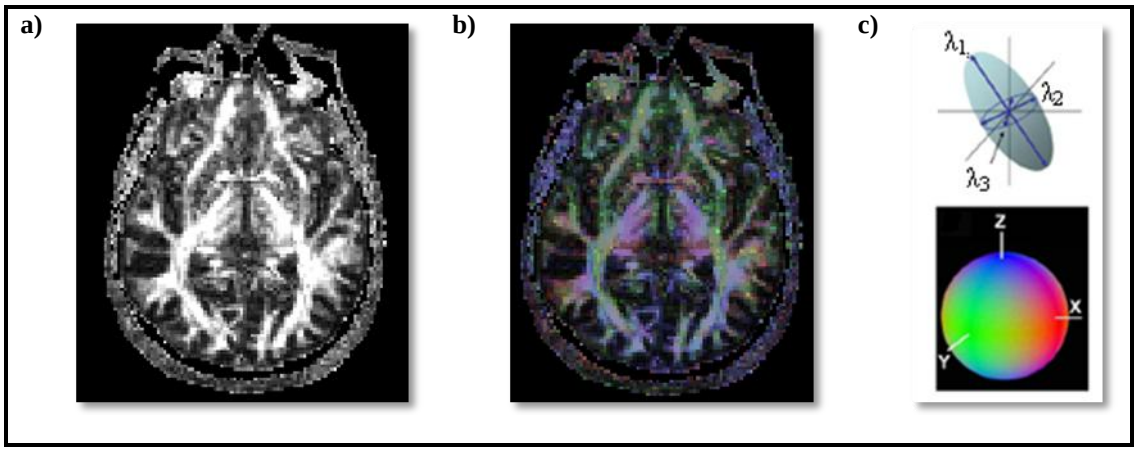


Figure 5-7: Colour Maps
a) FA map, each voxel gives a value which is the proportion of the diffusion tensor that is due to anisotropic diffusion, **b)** Eig X, Y and Z files can be overlayed onto the FA map to show directional dependence, **c)** The directions of the axis of the ellipsoid (top) correspond to the eigenvalues λ_1, λ_2 and λ_3 and each eigenvalue is represented by a different colour. Image from Mori & Zhang (2006).

The mean diffusivity map (*Figure 5-8*) shows the average speed and direction of movement through a voxel and can also be calculated from the eigenvector X, Y and Z files by averaging diffusivity along the principal axis (axial diffusivity) and each of the two minor axes (radial diffusivity) using the following formula:

$$MD = \frac{(\lambda 1 + \lambda 2 + \lambda 3)}{3}$$

where MD is mean diffusivity, λ1 is diffusivity in the X direction, λ2 is diffusivity in the Y direction and λ3 is diffusivity in the Z direction.

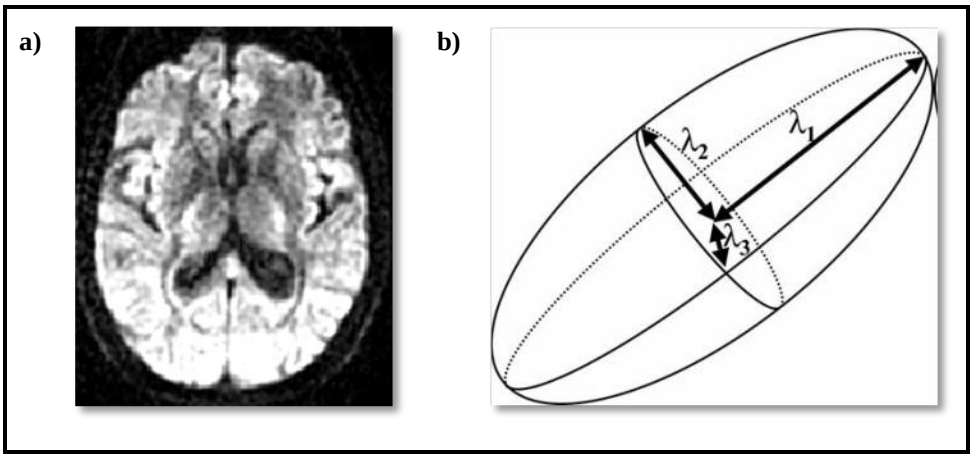


Figure 5-8: Diffusion weighted image
a) Image with diffusion weighting that is used to calculate mean diffusivity, **b)** diffusion (represented by an ellipsoid) can be measured by averaging diffusion in each direction. λ1 is the principal direction of diffusion with λ2 and λ3 diffusion in the minor axes. Each axes corresponds to the eigenvalues of the diffusion tensor (Watts, 2008).

In areas of greater diffusion (such as the ventricles), the pixels of the image are darker because as the signal is lost the molecule is free to move about. A higher diffusion value means the molecule has travelled far without interruption and it has taken the signal with it. Interpretation of MD values allows for inference about the integrity and density of cellular structures. A high MD value indicates that water movement is mostly unrestricted, inferring that cells are damaged in some way. A low MD value indicates cellular structures are mostly intact. Inferences about cell density can also be obtained from the MD values. MD is lower when cells are densely packed as there are more barriers against diffusion which results in a slower diffusion rate (Kinoshita, et al., 2008).

5.4 Summary

A convenience sample of PD patients and healthy control participants was collected from data held for ongoing research at the New Zealand Brain Research Institute, Christchurch. Several scanning sequences were performed on each individual which were incorporated into structural, clinical, blood flow and diffusion images. Patients and healthy control participants underwent a detailed neuropsychological examination that covered the four cognitive domains recommended by the Movement Disorder Society Task force (attention, executive function, learning and memory and visuospatial function). Patients received a cognitive diagnosis of either Parkinson's disease with dementia, Parkinson's disease with mild cognitive impairment or Parkinson's disease with no cognitive impairment. In order to include only healthy controls for comparison, those participants who met criteria for

MCI were also excluded. The final sample included 92 PD patients (PD-D = 17; PD-MCI = 19, PD-N = 56) and 25 control subjects.

Chapter 6. Study One: THE THALAMUS IN NATIVE SPACE

6.1 Objectives

The main objective of this chapter was to perform a novel investigation into the thalamic integrity of Parkinson's disease patients. A second objective was to determine if thalamic degeneration was primarily contributing to the cognitive, rather than the motor symptoms that characterise this disorder. This chapter was the first study conducted for this thesis and examines the thalamus in native subject space. Thalamic volume changes are investigated using structural T1 and thalamic microstructure using structural MD and FA images. Cross-sectional analysis was used to compare the three Parkinson's disease groups who were stratified by cognitive status and the healthy control group. The Parkinson's disease groups were PD with no cognitive impairment (PD-N), PD with mild cognitive impairment (PD-MCI) and PD with dementia (PD-D) (*Section 5.2*). It is assumed that any changes identified in those groups with worse cognitive dysfunction represent the progression of dementia development. Similarly, the comparison between PD-N and the healthy group is assumed to represent the changes that are primarily representative of Parkinson's symptoms and not cognitive dysfunction as this group exhibited neuropsychological test scores that were not consistently different from the control group. This chapter begins with a literature review of thalamic changes that have already been investigated in PD in native space. The characteristics of previous studies are outlined in *Table 6-1* and briefly discussed. Wider cortical changes identified in native subject space are also examined here as all cortical regions have strong connectivity with the thalamus and are likely to play a part in the degeneration of this region in PD (Kendi, Lehericy, Luciana, Ugurbil, & Tuite, 2008; Nagano-Saito, et al., 2005b).

6.2 Structural alterations in Parkinson's disease

Neuroimaging is a valuable tool used to measure the progression of disease related decline in PD by examining alterations in blood flow, glucose, oxygen and the metabolism of dopamine (Brooks, 2004; Monchi, Martinu, & Strafella, 2010). Lewy pathology and neuronal loss are not easily detected from neuroimages as degeneration must be substantial in order to be reflected in structural images (Stoessl, 2011). Structural examination is still valuable however, particularly in native subject space as it allows for the measurement of changes in individual patients and for *a priori* hypotheses to be made about areas that are

likely to be affected. Automated methods of segmentation are also available which reduces user bias, are more accurate and significantly faster than manual segmentation (Morey, et al., 2009). Structural imaging may also allow for pre-clinical identification of Parkinson's disease. Atrophy in the pons and medulla, identified using T1 imaging techniques (Jubault, et al., 2009), confirms the progression of degeneration as outlined in Braak's (Braak, Bohl, et al., 2006) model. Significant alterations are evident in these regions in patients who are in the very early stages (Hoehn and Yahr stage I and II) of disease. As the progression of disease correlates with cognition (Braak, Rub, et al., 2006) we can expect that other structural changes identified *in vivo* may also be reflective of cognitive impairment in PD.

Table 6-1: Previous literature which has addressed thalamic changes in PD in native subject space

Author	Cohort	Areas examined	Method of examination	Areas of volume loss
Volume changes				
In PD without dementia				
Bruck, Kurki, Kaasinen, Vahlberg, & Rinne, 2004	20 non-demented PD, 22 healthy age matched controls	prefrontal cortex (axial images), hippocampus (coronal images)	visual rating scale 0-4	bilateral prefrontal cortex, bilateral hippocampus PD < controls
Hu, et al., 2001	8 non-demented PD, 10 age matched controls	total brain, ventricles	registration of scans, visual rating scale, subtraction of follow up images from baseline images	total brain (visually identified to be worst in frontal, temporal and parietal regions), ventricles, PD < controls
Tinaz, et al., 2010	15 PD, 15 age matched controls	total brain, frontal, parietal, middle temporal, temporal pole, hippocampus, amygdala, thalamus, caudate, putamen, pallidum, accumbens	automated surface reconstruction and segmentation	Bilateral frontal gyrus, left sulcus. Bilateral putamen ratio PD < controls
Geng, Li, & Zee, 2006	16 early stage, 8 advanced stage PD, 8 normal controls	caudate, globus pallidus and substantia nigra	manual segmentation, completed twice and measurements averaged	putamen: PD < controls
Lisanby, et al., 1993	18 PD, 36 controls	thalamus, putamen, caudate	manual tracing from T2 images	thalamus, putamen, caudate: PD < controls
			6 month, 1 year, 2 year follow up	thalamus, putamen, caudate: PD < controls
Lee, et al., 2011	15 non-demented PD, 15 controls	thalamus, putamen and caudate nucleus	manual segmentation of ROI ‘	thalamus, putamen and caudate nucleus PD < controls
McKeown, et al., 2008	9 PD, 10 healthy, age-matched controls	thalamus	manual tracing and automated reconstruction	no volume change but areas of change within the thalamus thought to be the CM/Pf region PD < controls

Table 6-1 Continued

Peran, et al., 2010	30 early stage PD, 22 age, sex and education matched controls	thalamus, basal ganglia, substantia nigra	automated segmentation (FSL)	no change
Messina, et al., 2011	72 PD, 32 PSP, 15 MSA-P, 46 healthy, age matched controls	basal ganglia, limbic system	automated segmentation (FreeSurfer)	no change
Paviour, Price, Jahanshahi, Lees, & Fox, 2006b	18 PSP, 9 MSA-P, 9 PD, 18 healthy controls	frontal, midbrain, regions of the brainstem (pons, peduncles, cerebellum) and the lateral ventricles	semi-automated region of interest analysis	no areas of change
Paviour, et al., 2006a	17 PSP, 9 MSA-P, 9 PD, 18 healthy controls		12 month follow up	no areas of change
In PD with dementia				
Junque´, et al., 2005	16 PD, 16 PD-D, 16 age and education matched controls	hippocampus, amygdala	manual tracing, corrected for total brain volume	hippocampus, amygdala PD-D < controls
Camicioli, et al., 2011	23 non-demented PD, 10 PD-D, 39 controls	lateral, third ventricles (combined to give total ventricular volume)	manual segmentation of ROI '	total ventricular volume PD-D > PD
			36 month follow up	rate of change PD-D > PD
In PD with mild cognitive impairment				
Dalaker, et al., 2011	45 PD, 42 controls	thalamus, ventricles, caudate nucleus, pallidum, nucleus accumbens, hippocampus, amygdala, cerebellum	manual tracing from T1 image	left inferior lateral, third and fourth ventricles PD-MCI > C; third and left inferior lateral PD-MCI > PD.
Apostolova, et al., 2010	12 non-demented PD, 8 PD-MCI, 15 PD-D, 20 controls	ventricles, hippocampus, caudate nucleus	manual tracing from T1 image, converted into 3D average models	no change

Table 6-1 Continued

Cellular changes

In PD without dementia				
Peran, et al., 2010	same as above	same as above	same as above	thalamus mean diffusivity: PD > controls; thalamus fractional ainsotropy: PD < controls; substantia nigra mean diffusivity PD > controls.
Nicoletti, et al., 2006	16 PD, 16 MSA-P, 16 PSP, 15 controls	bilateral caudate, putamen, pallidus, thalamus, prefrontal and precentral white matter and middle cerebral peduncles	regions within ROI manually segmented	components of basal ganglia: no change; prefrontal and frontal white matter ADC: PD > controls
Schocke, et al., 2004	17 PD, 11 MSA-P, 10 healthy controls	basal ganglia (putamen, globus pallidus and caudate nucleus)	manual segmentation of ROI	globus pallidus rADC: PD > controls
Rizzo, et al., 2008	13 PD, 7 CBS, 10 RS, 9 controls	cerebral cortex, corpus callosum, putamen, midbrain, third ventricle, thalamus	manual segmentation of ROI	no change
Gattellaro, et al., 2009	10 non-demented PD, 10 age matched controls	substantia nigra, corticospinal tract, corticospinal tract in internal capsule, head of caudate nucleus, thalamus, globus pallidus, putamen, genu and splenium of corpus callosum, superior longitudinal fasciculus, cingulum	small regions within ROI manually defined	thalamus mean diffusivity: no change; substantia nigra mean diffusivity PD > controls. thalamus fractional ainsotropy: no change; superior longitudinal fasciculus, genu PD < controls.
Chan, et al., 2007	73 PD, 78 age and sex matched control subjects	thalamus, basal ganglia (caudate nucleus, globus pallidus, putamen, substantia nigra)	manual segmentation of ROI ‘s	thalamus FA and ADC: no change FA values of substantia nigra and putamen:PD < controls
Li, et al., 2010	14 depressed PD, 18 non depressed PD	thalamus	manual segmentation of thalamus	FA values of bilateral thalamus (mediodorsal region) PD depression < PD non depression; MD: no change

6.2.1 In Parkinson's disease without dementia

Focal volumetric reduction is mainly concentrated to regions within the frontal and temporal cortices in non-demented Parkinson's patients. Visual rating of cortical areas indicates reduction in the prefrontal (Bruck, Kurki, Kaasinen, Vahlberg, & Rinne, 2004) and frontal, temporal and parietal cortices (Hu, et al., 2001). Within the frontal lobe, segmentation of the frontal gyrus and sulcus after 3D reconstruction of the cortex also shows volume loss, without any change in total brain volume (Tinaz, et al., 2010). Subcortically, the bilateral hippocampus (Bruck, et al., 2004) and regions within the basal ganglia are the only regions to show significant volume reduction in non-dementing PD. The putamen and caudate nucleus, regions heavily involved in the motor loop (Braak & del Tredici, 2008) show significant atrophy when manually segmented from structural images in PD (Lee, et al., 2011a; Lisanby, et al., 1993). The putamen also exhibits significant levels of atrophy when it is examined independently from the rest of the basal ganglia using both automated (Tinaz, et al., 2010) and manual (Geng, Li, & Zee, 2006) segmentation methods.

In contrast, other studies that have applied semi automated and automated segmentation techniques have not detected any structural abnormalities in PD. Paviour, (2006b) isolated the frontal lobes, midbrain, lateral ventricles and regions of the brain stem (pons, peduncles, cerebellum) using semi-automated region of interest analysis and reported no volumetric reduction of any region. At 12 month follow up (Paviour, et al., 2006a) the PD patients continued to show normal regional volumes. Peran (2010) applied the same automatic segmentation technique we have used in this study and also report no atrophy of any regions within the basal ganglia or substantia nigra.

6.2.1.1 Thalamic changes in Parkinson's disease without dementia

The thalamus was included, or specifically targeted as a region of interest in some of the above studies. The level of thalamic involvement in non-dementing Parkinson's disease patients is controversial. Lisanby (1993) was the first to specifically target the thalamus as a region of interest in PD after recognising that the changes in the substantia nigra may lead to a breakdown in communication with connected subcortical nuclei. Thalamic volume was significantly reduced, and despite showing no correlation with disease duration continued to show reduction of 39.4%, 37.1%, and 35.9% in subsets of patients who had repeat MRI's at 6 months, 1 year or 2 years after their baseline assessment

respectively. Lee (2011a) later confirmed thalamic atrophy in PD, reporting significant atrophy in the thalamus and some basal ganglia regions compared to control subjects. Automatic segmentation of the basal ganglia and thalamus (Messina, et al., 2011; Peran, et al., 2009) gives conflicting results however. Both studies show no thalamic reduction, or reduction in any component of the basal ganglia in PD. A final study (McKeown, et al., 2008) which specifically targeted the thalamus may aid in understanding the discrepancy between these results as the authors report differential degeneration of a small area within the thalamus, but no overall volume loss. Alongside overall volume, the shape of the thalamus was also examined and a significant change was identified in the ventral region, thought to correspond to degeneration of the centromedian/parafascicular nucleus. Histology of this region has previously revealed significant Lewy body pathology, neuronal, and volumetric reduction which is concentrated to this area (Halliday, 2009). Examination of thalamic components, rather than the whole thalamus may therefore be more sensitive to PD dysfunction.

6.2.2 In Parkinson's disease with mild cognitive impairment

Structural changes in PD-MCI are not robust. To date, only two studies have quantified atrophy from T1 images in PD-MCI patients and report contrasting results in relation to ventricular dilation. One (Dalaker, et al., 2011) examined components of the limbic system, basal ganglia and the ventricles. Compared to control subjects, the left inferior lateral, third and fourth ventricles were all increased in PD-MCI. Within PD, the third and the left inferior lateral ventricle was increased in PD-MCI relative to PD. In contrast, Apostolova, et al., (2010) reported no ventricular changes in PD-MCI and changes only at trend level in other subcortical structures. Similar methodology was applied in both studies; the ventricles were manually segmented from T1 images in both cases but Apostolova and colleagues then converted these images into 3D group average models. In addition to the ventricles, this group also examined the hippocampus and caudate nucleus in this way. Although the hippocampus initially showed significant reduction in the PD-MCI group compared to the PD group, this effect disappeared after controlling for age.

6.2.2.1 Thalamic Changes in Parkinson's disease with mild cognitive impairment

Dalaker, et al., (2011) is the only study so far to include the thalamus for examination in PD-MCI in native space. Although the authors report changes in the ventricles surrounding the thalamus; the thalamus did not reflect increased levels of cognitive

impairment in this group. There was no atrophy in PD-MCI relative to controls or to PD without cognitive impairment.

6.2.3 In Parkinson's disease with dementia

In Parkinson's disease with dementia the focus has been on subcortical changes. Manual tracing methods have shown atrophy of the hippocampus and amygdala ratios (corrected for total brain volume) in PD-D relative to control subjects (Junque´, et al., 2005) and an increase in total ventricular volume in PD-D relative to non-dementing PD (Camicioli, et al., 2011). In the Camicioli, et al study, at 36 month follow up the rate of change was also significantly greater in PD-D compared to PD patients. Ventricular enlargement is particularly relevant as the ventricles surround grey matter structures and are directly affected by putamen and thalamic atrophy (Gaser, Nenadic, Buchsbaum, Hazlett, & Buchsbaum, 2004).

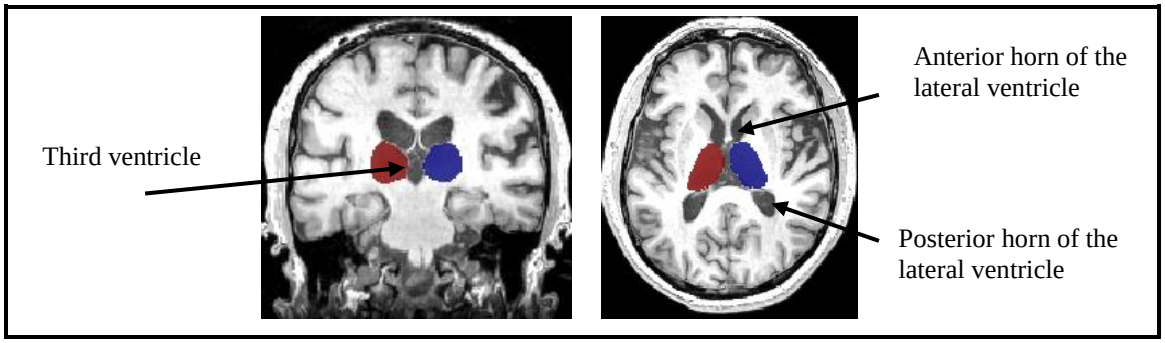


Figure 6-1: The third and lateral ventricles of the brain
Image is a T1 structural image of a Parkinson's disease patient with dementia who is a 61 year old male with 7 years of disease duration.

6.2.4 The relationship between cortical changes and cognitive dysfunction

In PD without dementia, atrophy is mainly evident in regions that correlate with movement dysfunction such as the basal ganglia or substantia nigra. Degeneration of the frontal and temporal regions, particularly in the hippocampus could be representative of cognitive dysfunction however. In the Hu, et al., (2001) sample, for example, volume reduction of the whole brain was associated with estimated reductions in performance IQ (estimated by subtracting IQ at follow up from IQ at baseline as measured by the National Adult Reading Test-Revised). At two year follow up these patients had lost further brain volume at a rate that was significantly greater than the control subjects. The level of atrophy was directly related to declining cognition as at follow up, patients were further impaired on tests of vocabulary, visuospatial ability, memory and general cognition and there was a strong

correlation with the percentage of brain loss. This study is particularly relevant as findings remained even after accounting for motor symptoms. In the only other study to examine the influence of atrophy on cognitive dysfunction in non-dementing PD, Bruck (2004) performed correlation analysis between level of atrophy and cognition. The left hippocampus was correlated with verbal memory and prefrontal atrophy correlated with attention impairment. The association between the level of atrophy and cognition may be particularly relevant in this early stage of Parkinson's disease as attention and memory dysfunction are some of the key deficits identified in early stage PD patients who later go on to develop dementia (Janvin, et al., 2006).

The hippocampus was also implicated in Parkinson's disease with dementia (Junque´, et al., 2005), indicating degeneration in this region is likely to be progressive and worsen in accordance with cognitive dysfunction. There was a significant association with both the amygdala and hippocmapus with global cognition in this sample and with the hippocampus and delayed recall. Ventricular enlargement is also reflective of cognitive dysfunction in Parkinson's disease dementia, showing a significant association with Dementia Rating Scale Scores and the Mini Mental State Exam but not the UPDRS score in PD-D (Camicioli, et al., 2011).

In PD-MCI, only the fourth ventricle, located in the brainstem has an association with cognition and shows a high correlation with memory performance (Dalaker, et al., 2011). This finding could reflect Braak's (2006) model of disease progression as the lower levels of the brainstem regions are infiltrated first. Ventricle association with memory impairment here could therefore be reflective of the greater cell loss in surrounding brain stem regions that has not yet occurred in higher regions. The ventricles were also the only regions implicated in relation to cognitive dysfunction in the Apostolova, (2010) study and were significantly correlated with the MMSE, despite not showing significant levels of enlargement in the PD group. More sensitive examination of the regions surrounding the ventricles could therefore reveal significant alterations and a strong association with early stages of cognitive decline in Parkinson's disease.

6.2.5 Cellular changes

Neuronal loss must be severe to induce atrophy in subcortical regions (Halliday, 2009) and atrophy is not easily detected from structural MR images (Brooks, 2010). Diffusion tensor imaging (DTI), on the other hand may allow for visualisation of neuronal degeneration or integrity disruption before gross atrophy is visible. One sample (Peran, et al., 2009) to date

has shown cellular changes in the thalamus, reflected in altered DTI measures but in the absence of atrophy in Parkinson's disease. Despite no evidence of volumetric changes, the thalamus showed significantly higher mean diffusivity and lower fractional anisotropy in the PD group compared to the control group. Although the basal ganglia was also examined, the only other region to show cellular changes was the substantia nigra which showed reduced FA in the PD group. Combined with the parameters of the substantia nigra, the diffusion measures of the thalamus showed a good sensitivity (100%) and specificity (80%) for the classification of Parkinson's disease patients.

Other studies (Nicoletti, et al., 2006; Rizzo, et al., 2008; Schocke, et al., 2004) have also applied DTI measures in the thalamus. This technique has mainly been used to investigate the validity of using cellular changes as a tool for differential diagnosis of other parkinsonism disorders which have a similar presentation of motor symptoms. Although Nicoletti, et al., reports increased diffusivity in the prefrontal cortex and Schocke, et al., reports increased diffusivity in the globus pallidus of PD subjects all three studies show no diffusivity changes in the thalamus relative to control subjects. Two other studies (Chan, et al., 2007; Gattellaro, et al., 2009) have also examined the thalamus in early stage PD using DTI techniques with both reporting no cellular abnormalities although there was increased MD (Gattellaro, et al., 2009) and reduced FA (Chan, et al., 2007) in the substantia nigra. The only other study (Li, et al., 2010) to examine the thalamus in PD reports reduced FA in the mediodorsal region of the thalamus of PD patients with co-morbid depression relative to PD patients without depression. None of the above diffusion studies examined cellular abnormalities in relation to cognitive dysfunction which leaves significant questions surrounding the influence of the thalamus on the cognitive symptoms of PD.

6.3 Overcoming the limitations of previous work

Cognition was only explicitly examined in a few of the above studies (Gattellaro, et al., 2009; Li, et al., 2010; Nicoletti, et al., 2006; Peran, et al., 2010). The majority limited testing to the MMSE only (Li, et al., 2010; Nicoletti, et al., 2006; Peran, et al., 2010) or diagnosed PD-D based on the MMSE and DSM criteria for dementia which is not specific to Parkinson's disease dementia (Gattellaro, et al., 2009). It is difficult to quantify the relationship between the thalamus and cognition in Parkinson's disease from what has been conducted so far.

Very few studies have examined the thalamus in Parkinson's disease. From those that have, there are several limitations. The main limitation is that few neuropsychological evaluations have been carried out in all previous studies that have included the thalamus as a region of interest in PD cohorts. In many cases cognitive dysfunction is present even in the early stages of PD (Janvin, Larsen, Aarsland & Hugdahl, 2006) and may be evident at subthreshold levels in these samples.

Methodology is also another limiting factor as only two studies (Li, et al., 2010; McKeown, et al., 2008) identified the thalamus as a region of interest *a pirori*, problematic when there is no provision made for multiple comparisons. There is also the issue of sample size: in two cases (McKeown, et al., 2008; Nicoletti, et al., 2006), the sample size was less than 20. Small sample size greatly reduces the power of a study and increases Type II error, a phenomenon where the effect exists, but is not detected in statistical analysis (Howell, 2007).

To overcome these common problems this research used a large sample size, participants received extensive neuropsychological testing and confounding effects such as age and clinical symptoms such as motor dysfunction were controlled for in statistical analyses. In addition, all PD patients were diagnosed as either PD with dementia, PD with mild cognitive impairment, or PD with no detected cognitive impairment. This provided a cross section of cognitive function within PD and enabled in depth examination of thalamic degeneration at various disease stages.

6.4 Summary

The structural changes in areas that are connected to, or are adjacent to the thalamus such as the frontal and temporal lobes (Hu, et al., 2001) and the structures of the basal ganglia (Geng, et al., 2006), limbic system (Junque´, et al., 2005) and ventricles (Camicioli, et al., 2011) which show a relationship with cognition suggest that the thalamus will also be reflective of cognitive dysfunction in PD. The ventricles are particularly relevant as ventricular enlargement is the only region to date that shows a significant change between PD with and without MCI (Dalaker, et al., 2011). Thalamic change therefore could also be a marker of disease progression and aid in identifying PD-MCI and separating these patients from those without impairment.

To date, thalamic changes have been identified in some PD cohorts. Volumetric analysis has shown a trend for decreased volume in PD (McKeown, et al., 2008), while the diffusion measure of mean diffusivity shows a trend for higher values in patients (Nicoletti,

et al., 2006; Rizzo, et al., 2008). In a PD patient sample with intact cognition (Li, et al., 2010), there was no difference in MD values of the thalamus. Microstructural changes appear to be more sensitive to symptoms in the earliest stages of PD as both a reduction in FA and increase in MD in the thalamus of a PD group has been identified in the absence of volume reduction (Peran, et al., 2010).

6.5 Hypothesis

- That the whole thalamus will be reflective of cognitive dysfunction in Parkinson's disease

6.6 Method

6.6.1 Participants

The thalamus was able to be automatically segmented from all images so all participants (*Section 5.2*) were included in this initial study. The final sample of participants for which the thalamus was defined included 56 PD-N, 19 PD-MCI, 17 PD-D patients and 25 healthy control participants.

6.6.2 Image Preparation

Before image analysis could be conducted, the raw structural images had to undergo a number of different prepatory procedures. The following steps were all completed using the Statistical Parametric Mapping 5 (SPM 5) programme (Friston, 2005).

6.6.2.1 Reslicing the T1 image

The T1 image was resliced to fit the FA image (rT1) so that the voxels of the T1 image were the same size as the voxels in the FA image (1.88 x 1.88 x 3mm^3). This is a necessary step to ensure the FA image can be overlayed onto the T1 image which aids in the manual definition of thalamus boundaries. The 'realign: reslice' option was used and the FA image was entered first as the reference image and the T1 second as the image to be resliced. Images were resliced using the 2.n option, which only reslices the second image in the list, leaving the first intact. Interpolation for writing the T1 image to the FA space was defined as nearest neighbour as this was the most suitable for our images. Images were not wrapped to fit the defined space and were left in standard subject (native) space.

6.6.2.2 Orientation to the Anterior Commissure

Images were orientated to the Anterior Commissure (AC) in order for all the images created from one individual to have the same MNI co-ordinates. SPM allows for the structural T1 image to be displayed in all three planes (X, Y, Z) simultaneously (*Figure 6-2*), enabling the manual realingment of the image by the user (N.B). This is done using the pitch (X axis), roll (Y axis) and yaw (Z axis) function which are defined on a trial and error basis until the image is alinged upright and straight in all three views. When the image is aligned to a satisfactory degree, the anterior commissure (AC) is isolated and set at position 0,0,0. All other scans are then registered to the same position using T1 as the template using SPMs 'coreg: estimate' option, ensuring that the X,Y, Z co-ordinates of one

image correspond to the same place in the brain of the X,Y,Z co-ordinates on all other images.

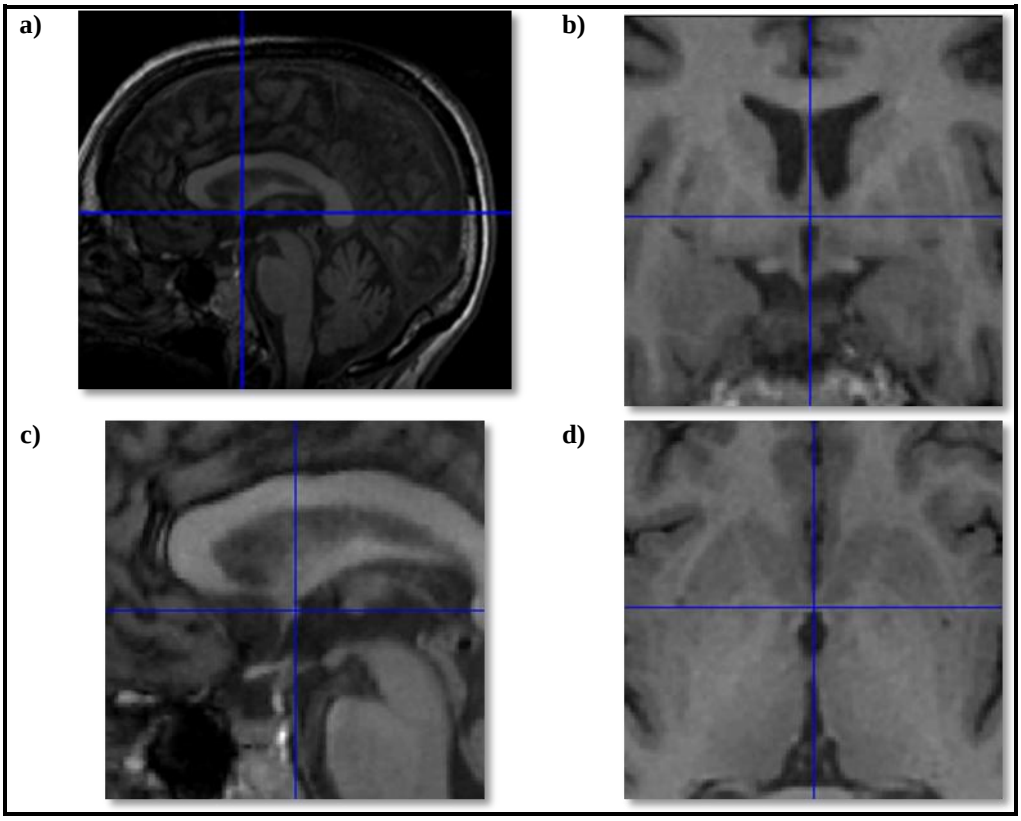


Figure 6-2: Orientation to the anterior commissure
The location of the anterior commissure is presented on the whole brain saggital (**a**) view and corresponding 4x magnified coronal (**b**), saggital (**c**) and horizontal (**d**) views. The SPM 5 programme is employed to orientate all scans to the anterior commissure

6.6.2.3 Masking

For 8 participants (seven patients and one control subject) assistance was needed to keep the head still during the scanning procedure. For these individuals only, a styrofoam gel pad was placed in the scanner and aided in the correct positioning of the patients head. Because the FIRST programme uses a probability map based on voxel intensity to define the thalamus and the gel pad appears as a solid white mass on the images, the presence of the gel pad prevents accurate definition of the thalamus in these cases. The gel pad was removed from each image by the manual creation of a brain mask. A pen tablet was used to enable the user (N.B) to manually trace onto the image, and all white voxels corresponding to the gel pad were isolated. The tracing defined a region of interest that could then be excluded from the image using the 'mask' function of MRIcron's draw tools. The new T1 image was saved and used for all future processing.

6.6.3 Image Analysis

The algorithm FIRST (v 1.2) was applied to separately estimate left and right volume of the thalamus from the T1 image in native space. FIRST is part of FMRIB's Software library (FSL: Patenaude, 2007) and performs both the registration and segmentation of the thalamus, transforming the image to MNI standard space (*Figure 6-5*). Following recommendations of de Jong et al.,(2008) a boundary correction of a Z-value of 3 was used, corresponding to 99.98% certainty that voxels belonged to the thalamus. Accuracy was confirmed by visual inspection of all MR images and comparison to anatomical guidelines (Duvernoy, 1991; Nolte & Angevine, 2007). In addition, a sub set (n = 7) was manually traced for comparison purposes. The manual and automated methods were compared using a *t* test for dependent samples. The different methods did not result in a difference between thalamus size for either the left [$t(6) = -0.78$, $p = 0.47$] or right, [$t(6) = -0.94$, $p = 0.38$] thalamus.

6.6.3.1 Manual Tracing – The Gold Standard

The thalamus was manually segmented based on the guidelines of Portas, et al., (1998) who defined boundaries according to the anatomical guidelines of (Duvernoy, 1991). For reference another more detailed anatomical atlas was also used (Nolte & Angevine, 2007). Manual tracing of the thalamus was achieved using the MRICRON programme which allows the user to view the image in coronal, saggital and axial planes simultaneously and use the pen tablet. The boundaries of the thalamus are, for the most part, easily visualised due to the high contrast between grey matter and the cerebral spinal fluid which fills the ventricles. All borders were manually traced onto the resliced T1 image (rT1) image as this provided the highest level of contrast (*Figure 6-3*). The FA maps, coloured to show directional dependence are used to define the internal capsule which forms the lateral boundary. When the 'BLUE' file (superior-inferior direction) is overlayed onto the rT1 image the internal capsule appears as a solid blue line, allowing for easy identification of this border. This is best seen on the axial image although it is useful to simultaneously check segmentation on the coronal image. The medial boundary is defined by the third ventricle and the dorsal boundary by the main body of the lateral ventricle. The inferior boundary is not as easily defined as it must be segmented from the brainstem. This is best done on the saggital image as there is sharp contrast between white matter of the brainstem and grey matter of the thalamus in this image.

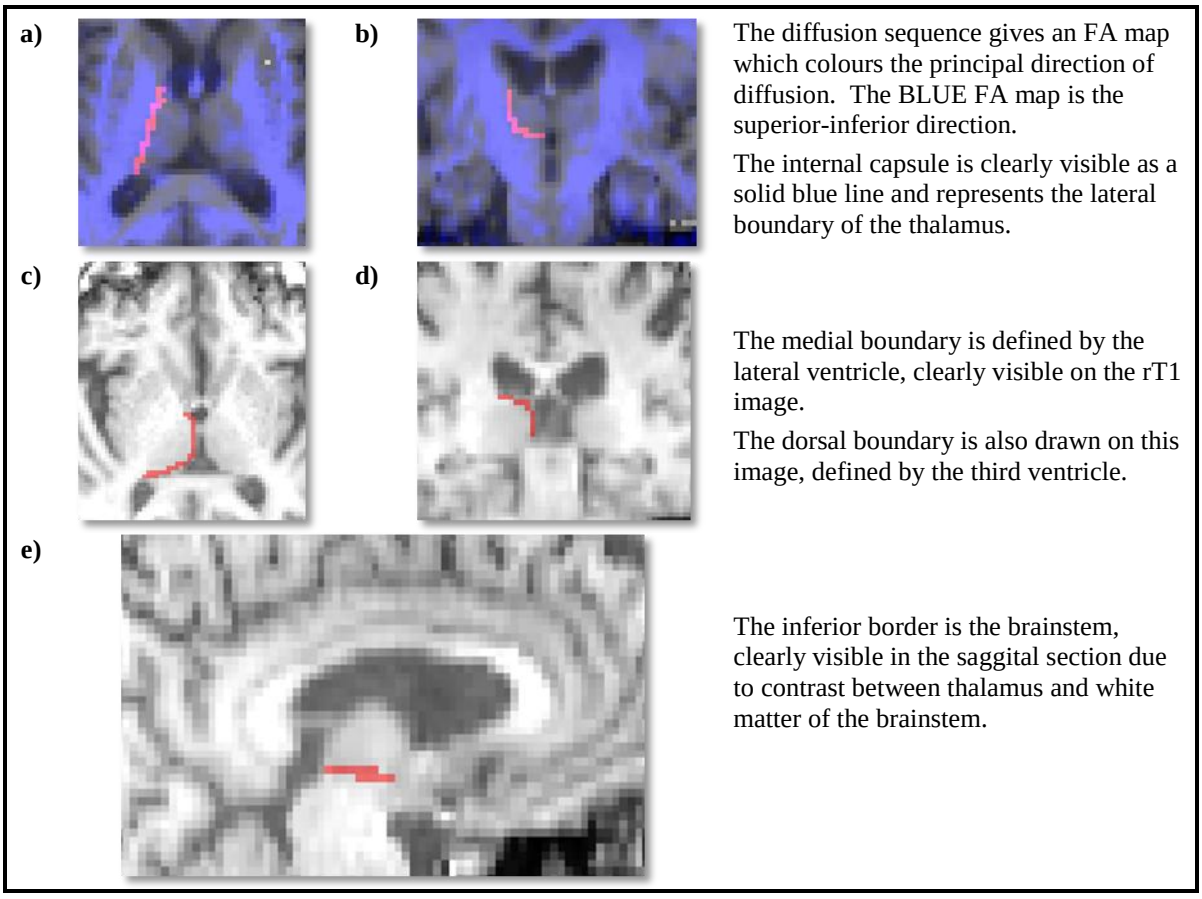
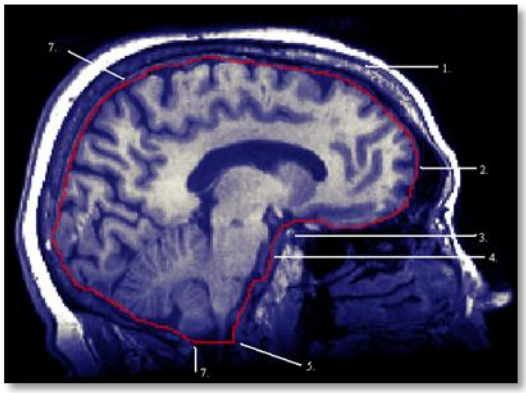


Figure 6-3: Thalamus Boundaries
The boundaries used for the manual definition of the thalamus are easily visualised after application of the directional 'BLUE' image (**a, b**) while the dorsal (**c**) and medial (**d**) boundaries are easily visualised on the rT1 image due to the contrast with ventricles. Definition of the ventral boundary is more difficult due to merging with the brain stem, but the saggital image (**e**) provides the best view and contrast between the grey matter of the thalamus and white matter of the brain stem.

Intracranial volume was measured from the T1 image by one observer (N.B.) following the method of (Eritaia, et al., 2000). The intracranial cavity of each 10th saggital slice was manually traced using the dura matter as a border. The first slice was randomly selected within the first 10 slices that the cortex became visible. In the absence of dura matter the cerebral contour is outlined. Other landmarks include the undersurface of the frontal lobe in which pixels deemed to be cerebral cortex are rendered until a white line is visible, this is the dorsum sellae. This line is then employed as a guide to the cilvus, which appears as a solid white dot. The intracranial cavity includes the CSF to the left of this landmark and rendering excludes any white pixels. When the base of the brain is reached, a horizontal line must be drawn between the anterior and posterior arch of the first cervical vertebra (C1) to segment the cortex from the brainstem. The last saggital slice occurs within the last 10 slices that cortex is visible. ICV is then calculated using MATLAB which multiplies the total of all slices by 10 to give overall volume. For each subject the

ROI is then able to be divided by that persons intracranial volume, giving an accurate ratio of the region of interest relative to total cranial size.



1. The outer table of the skull.
2. Cerebral contour.
3. The dorsum sellae.
4. Clivus.
5. Anterior arch of C1.
6. Posterior arch of C1.
7. Dura matter, visible as a white line.

Figure 6-4: Boundaries for the manual segmentation of intracranial volume
The red line indicates the region of interest, manually outlined in the MRIcron programme. Also note that although the cerebral cortex shows signs of atrophy this does not affect the location of the dura matter.

6.6.3.2 Automatic Segmentation

The FSL programme provides comprehensive tools that can be used with fMRI, MRI and DTI imaging data. For the purposes of this research the FSL FIRST v 1.2 tool was used to define the left and right thalamus of each individual. The FIRST tool is FMRIB's integration and segmentation tool which segments subcortical brain structures using Bayesian shape and appearance models (Patenaude, Smith, Kennedy, & Jenkinson, 2011).

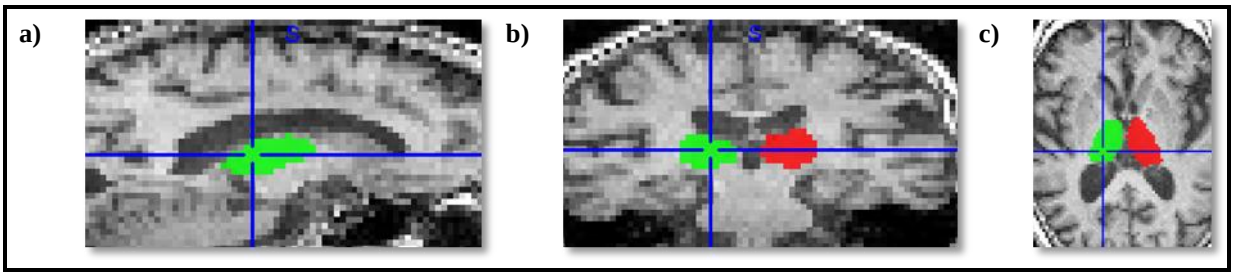


Figure 6-5: Automatic Segmentation of the Thalamus
a) Saggital X = 57, **b)** Coronal Y = 58 and **c)** Horizontal Z = 27 sections of FIRST segmentation of left (red) and right (green) thalamus overlayed on the rT1 image.

The individual T1 image data to be analysed is uploaded and converted to native space. The thalamus was then determined based on a large database of hand-segmented images provided by Centre for Morphometric Analysis (CMA), MGH, Boston. FIRST searches through linear combinations of shape modes for the most probable shape given the intensities of the T1 image and then renders the voxels that are determined to be a part of that structure. The entire sequence takes approximately 25 minutes per scan and gives

the user individual images for each subcortical structure. Once thalamus is defined the image file is created and can be read by MATLAB to give total volume.

6.6.4 Diffusion Weighted (MD and FA) Imaging

The diffusion variables are calculated from the mean diffusivity and fractional ainsotropy maps. The whole image file is imported into MATLAB and values read for each voxel of the image. In order to limit values to thalamus only, the automatically defined thalamus is then imported into Matlab and only those values which fall within these voxels are read. An average value FA and MD value is calculated for each thalamus.

6.6.5 Statistical Analysis

Between group differences were examined using a between and within groups analysis of variance (ANOVA) where the dependent variable in each model was the integrity measure of the thalamus (volume, FA or MD) and the independent variable the subject group (healthy control, PD-N, PD-MCI and PD-D). The post hoc Newman-Keuls analysis was applied in the case of a significant group effect. In the case of a significant group by hemisphere interaction, a one way ANOVA was conducted independently for each hemisphere if there was significant lateralisation of the group effect. Where required, Kruskal-Wallis ANOVA was employed for non parametric data. Analysis of covariance was used to test the effects of the covariates: age; education and depression. Separate models were used in which control participants were excluded and the additional continuous predictors (disease duration and UPDRS) included as co-variates to test the effects of clinical covariates specific to PD.

The relationship between the thalamus and cognition was tested by a series of Pearson correlations in the first instance. In the case of a significant relationship, multiple regression analysis was employed to test the effect while accounting for covariates. In the first regression model the thalamic variables MD, FA and volume were entered as independent variables along with the demographic (age, sex, education, depression) and clinical (disease duration) variables to examine the relationship with global Z score. Additional multiple regressions were applied in the same way in order to predict score in the other four domains: attention/working memory/processing speed; executive function; visuospatial/visuopercetption and learning and memory.

Receiver operating characteristics (RoC) analysis was used to distinguish the cognitively impaired groups from those not impaired, and in some cases those less

impaired using the thalamic variables (e.g.: the following pairwise comparisons could be examined: C>PD-N>PD-MCI>PD-D).

6.7 Results

6.7.1 Participant information

Demographic and clinical characteristics for all participants are reported in *Table 6-2*. Adjacent group wise comparisons show that the PD-MCI ($p = 0.04$) and PD-D ($p < 0.01$) groups are older and have lower levels of premorbid IQ than the PD-N group. The PD-N group did not differ from the healthy control group on any measures. As expected, there was progressive worsening of clinical function and length of disease duration from PD-N to PD-MCI (Hoehn and Yahr $p < 0.01$; UPDRS III $p = 0.03$; disease duration $p < 0.001$) and from PD-MCI to PD-D (Hoehn and Yahr $p < 0.001$; UPDRS III $p < 0.001$; disease duration $p = 0.03$).

Measures of global cognition, cognitive domain scores and individual neuropsychological test scores are reported in *Table 6-3*. The PD-N group was comparable to the control group, only exhibiting lower performance on the map search, category switching, trails, the Stroop interference task, CVLT and RCFT measured delayed memory and aggregate Z scores of executive function, learning and memory and general cognition. The PD-D group was significantly worse on all tasks compared to the PD-N group and the PD-MCI group performed at a level consistently better than that of the PD-D group and lower than that of the PD-N group.

Table 6-2: Mean (std dev) of demographic and clinical data for all participants

	Control (n = 25)	PD-N (n = 56)	PD-MCI (n = 19)	PD-D (n = 17)	Statistic	p	Adjacent pair-wise comparisons
Demographic							
Age	67.2 (9.6)	64.7 (8.4)	70.8 (8.5)	73.4 (6.8)	F=5.78	<0.01	C=N<MCI=D
Education	13.8 (2.8)	13.5 (3.0)	12.2 (3.3)	12.4 (2.3)	F=1.40	0.25	C=N=MCI=D
Depression (GDS) [median (range)]	0.00 (0-1)	0.0 (0-9)	0 (0-11)	4 (0-8)	H=15.58	<0.01	C=N=MCI=D
Sex	17/8	37/19	13/6	15/2	χ^2=3.18	0.36	
Handedness	20/4	56/2	19/3	16/1	χ^2=5.19	0.16	
Premorbid IQ (WTAR)	117.8 (1.0)	114.4 (8.5)	107.2 (12.5)	106.1 (11.5)	H=17.38	<0.01	C=N>MCI=D
Clinical							
Hoehn + Yahr [median (range)]		2.0 (1-3)	2.5 (1.5-4)	4 (2-4)	F=28.74	<0.01	N<MCI<D
UPDRS III (max=88)		24.9 (13.6)	34.7 (16.4)	52.9 (20.1)	F=25.85	<0.01	N<MCI<D
Disease Duration		3.9 (3.3)	7.5 (5.3)	12.9 (8.5)	H=21.91	<0.01	N<MCI<D

GDS: Geriatric depression scale; **WTAR:** Weschler test of adult reading; **UPDRS III:** Unified Parkinson's disease rating scale. All participants completed all tests unless otherwise indicated by n value.

Table 6-3: Mean (std dev) of neuropsychological test scores for all participants

	Control (n = 25)	PD-N (n = 56)	PD-MCI (n = 19)	PD-D (n = 17)	Statistic	p	Adjacent pair-wise comparisons
Global Cognitive							
MMSE (max=30)	28.6 (1.5)	28.5 (1.4)	26.1 (2.4)	22.9 (3.2)	H=28.2	<0.01	C=N>MCI>D
MoCA (max=30)	27.8 (1.5)	26.7 (2.1)	22.6 (2.3)	16.4 (3.9)	H=38.2	<0.01	C=N=MCI>D
Attention and working memory^{+}	0.4 (0.5)	-0.0 (0.4)	-0.9 (0.5)	-2.0 (0.6)	F=98.7	<.001	C>N>MCI>D
Digits Forward/Backwards	1.0 (1.2)	0.4 (0.8)	0.0 (0.9)	-0.8 (1.0)	F=13.4	<.001	C=N>MCI>D
Digit Ordering	-0.4 (0.9)	-0.5 (1.0)	-1.7 (0.7)	-2.2 (0.6)	F=24.8	<.001	C=N>MCI>D
Map Search	0.7 (1.0)	-0.2 (0.9)	-1.7 (0.7)	-2.3 (0.8)	F=50.5	<.001	C>N>MCI>D
Stroop Colour Trial	0.2 (0.8)	-0.0 (0.9)	-0.6 (0.8)	-2.0 (1.0)	F=29.0	<.001	C=N>MCI>D
Stroop Word Trial	0.3 (0.6)	0.1 (0.6)	-0.5 (0.8)	-1.7 (1.2)	H=36.5	<.001	C=N>MCI>D
Trail Making Test A	0.3 (0.9)	0.0 (0.7)	-0.8 (1.0)	-2.7 (0.5)	F=65.0	>.001	C=N>MCI>D
ExecutiveFunction^{+}	0.9 (0.5)	0.4 (0.6)	-0.7 (0.7)	-2.1 (0.5)	F=101.6	>.001	C>N>MCI>D
Letter Fluency	1.1 (1.2)	0.8 (1.1)	-0.2 (1.1)	-1.5 (1.0)	F=22.6	>.001	C=N>MCI>D
Action Fluency	0.7 (1.0)	0.2 (1.3)	-0.9 (1.0)	-1.7 (0.7)	F=21.7	>.001	C=N>MCI>D
Category Fluency	1.2 (1.0)	0.9 (1.2)	-0.4 (1.0)	-2.1 (0.9)	F=32.7	>.001	C=N>MCI>D
Category Switching	1.1 (0.9)	0.3 (0.9)	-0.7 (0.8)	-2.1 (0.9)	F=55.4	>.001	C>N>MCI>D
Trail Making Test B	0.7 (0.5)	0.0(0.9)	-0.9 (1.2)	-2.9 (0.3)	H=55.8	>.001	C>N>MCI>D
Stroop Interference	0.6 (0.5)	0.4 (0.7)	-1.2 (1.5)	-2.5 (0.8)	H=56.1	>.001	C=N>MCI>D
Learning and memory^{+}	0.9 (0.8)	0.4 (0.8)	-0.7 (0.5)	-1.7 (0.7)	F=55.6	>.001	C>N>MCI>D
CVLT Free Recall	0.9 (0.9)	0.5 (0.8)	-0.9 (0.9)	-2.0 (1.0)	F=48.3	>.001	C=N>MCI>D
CVLT Short Delay (30s)	1.1 (1.2)	0.4 (1.1)	-0.7 (1.0)	-1.8 (0.8)	H=50.3	>.001	C>N>MCI>D
CVLT Long Delay (10min)	0.8 (0.8)	0.4 (0.8)	-0.5 (0.8)	-1.0 (0.7)	F=23.3	>.001	C=N>MCI>D
RCFT Immediate (3 min)	1.0 (1.3)	0.3 (1.4)	-0.5 (1.4)	-1.8 (0.8)	F=17.1	>.001	C=N>MCI>D
RCFT Delayed (30 min)	0.8 (1.5)	-0.3 (1.5)	-1.2 (1.2)	-1.9 (1.1)	F=13.5	>.001	C>N=MCI=D
Visuospatial/Perception function^{+}	0.6 (0.5)	0.5 (0.4)	-0.4 (0.6)	-1.3 (0.8)	H=46.1	>.001	C=N>MCI>D
RCFT Copy	0.3 (0.9)	0.1 (0.8)	-0.9 (1.3)	-2.0 (1.3)	F=25.7	>.001	C=N>MCI>D
JOL	0.7 (0.6)	0.6 (0.6)	-0.1 (0.9)	-0.8 (1.0)	F=23.0	>.001	C=N>MCI>D
VOSP Fragmented Letters	0.8 (0.8)	0.7 (0.5)	-0.0 (1.1)	-1.0 (1.1)	F=24.8	>.001	C=N>MCI>D
Global Z^{+}	0.7 (0.4)	0.3 (0.4)	-0.7 (0.4)	-1.7 (0.5)	F=165.5	>.001	C>N>MCI>D

+age adjusted Z score. Global Z score is an aggregate score of the mean value in each cognitive domain. **MMSE:** Mini mental state examination; **MoCA:** Montreal cognitive assessment. **CVLT:** California verbal learning test; **RCFT:** Rey complex figure test; **JOL:** Judgement of line orientation; **VOSP:** Visual object space perception battery. All participants completed all tests unless otherwise indicated by n value. Three participants (PD-N) were not tested on the MoCA; there was no other missing data

6.7.2 Thalamic volume

The thalamus volume was examined as an absolute value and as a ratio, relative to the individuals intracranial volume (thalamus divided by ICV for each subject). Between – within ANOVA examined group differences and the co-variates age, education and depression added in ANCOVA model. A subsequent ANCOVA model which excluded the control subjects and additionally included disease duration and UPDRS III examined the group effect of thalamic degeneration after accounting for clinical co-variates. The assumption of normality was tested using the Shapiro-Wilk test, and held for all thalamic variables in all four groups. Intracranial volume in the dementia group deviated slightly from normal (SW-W = 0.87, $p = 0.02$) due to a slight positive skew (skew = 1.48). Homogeneity of variance, examined using the Levene's Test held for all variables. The assumption of independence was upheld by using the left/right thalamic variables in separate analysis to the weighted average or total thalamic variables and correcting for multiple comparisons as necessary. As the deviations from normality were slight and all other assumptions held, statistical analysis continued as normal and non parametric testing was not needed. In regards to the ANCOVA models, the additional assumption of linearity - that the relationship between Y and each co-variate is linear was also necessary. This assumption held for all groups.

Mean values of all thalamic measures are shown in *Table 6-4* with adjacent group comparisons after accounting for co-variates presented in *Figure 6-7*. For absolute thalamic volumes and ICV there were no group differences. The left hemisphere was larger than the right [$F(1,113) = 79.50$, $p < 0.01$] but this did not result in a group by hemisphere interaction [$F(3,113) = 1.45$, $p = 0.23$]. Adding demographic covariates to the model did not result in a group effect [$F(3,110) = 1.03$, $p = 0.38$] but the effect of hemisphere was no longer significant [$F(1,110) = 2.55$, $p = 0.11$]. Excluding the control subjects in the second model and adding clinical covariates did not change results [$F(2,84) = 0.54$, $p = 0.58$].

Correcting for intracranial volume changed results; the PD groups were sensitive to thalamic degeneration. Thalamic volume was significantly reduced in the dementia group relative to the control subjects ($p = 0.03$). As with the absolute values, the hemisphere effect remained [$F(1,113) = 80.25$, $p < 0.01$] but this was a consistent effect across all groups and there was no interaction [$F(3,113) = 1.00$, $p = 0.40$]. When demographic covariates were added to the model the volume in the dementia group was significantly

lower than all other groups (*Figure 6-6*) and the hemisphere effect was no longer significant [$F(3,110) = 1.10$, $p = 0.35$]. Adding clinical covariates in the second model did not change results [PD-N $p = 0.03$; PD-MCI $p = 0.01$].

Table 6-4: Comparison of thalamic integrity measures between PD groups (stratified by cognitive status) and control subjects

	C (n = 25)	PD-N (n = 56)	PD-MCI (n = 19)	PD-D (n = 17)	Group Effect		
					F	p	Newman-Keulis
Volume (mm^3)							
Absolute	6721.80 (637.4)	7030.12 (760.21)	6666.52 (682.1)	6794.57 (962.7)	1.45	0.23	C=N=MCI=D
Left	6824.82 (674.7)	7168.16 (790.95)	6752.81 (643.1)	6960.8 (1012.2)	1.93	0.13	C=N=MCI=D
Right	6618.78 (626.7)	6892.09 (753.8)	6580.24 (743.4)	6628.35 (927.8)	1.38	0.25	C=N=MCI=D
Relative x 10^{-3}	4.52 (0.4)	4.53 (0.4)	4.53 (0.4)	4.27 (0.4)	2.23	0.09	C>D
Left	4.59 (0.4)	4.62 (0.4)	4.59 (0.4)	4.37 (0.4)	1.76	0.16	C=N=MCI=D
Right	4.45 (0.4)	4.45 (0.4)	4.47 (0.4)	4.17 (0.4)	2.61	0.05	C=N=MCI>D
ICV (cm^3)	1492.1 (149.0)	1557.4 (181.1)	1472.8 (100.0)	1593.0 (177.5)	2.56	0.06	C=N=MCI=D
Fractional Ainsotropy (range 0-1)							
Left	0.32 (0.02)	0.32 (0.03)	0.32 (0.03)	0.32 (0.02)	0.04	0.99	C=N=MCI=D
Right	0.31 (0.02)	0.31 (0.03)	0.30 (0.02)	0.31 (0.02)	0.37	0.77	C=N=MCI=D
Weighted average	0.31 (0.02)	0.31 (0.02)	0.31 (0.02)	0.31 (0.01)	0.16	0.92	C=N=MCI=D
Mean Diffusivity (mm^2 per sec x 10^{-3})							
Left	0.88 (0.05)	0.88 (0.05)	0.91 (0.06)	0.95 (0.06)	8.84	0.00	C=N<MCI<D
Right	0.88 (0.05)	0.89 (0.05)	0.92 (0.06)	0.95 (0.05)	8.23	0.00	C=N<MCI<D
Weighted average	0.88 (0.04)	0.88 (0.05)	0.92 (0.06)	0.95 (0.05)	9.66	0.00	C=N<MCI<D

Absolute and relative (thal/ICV) thalamic volume, fractional anisotropy and mean diffusivity measures are reported mean (SD). Repeated measures ANOVA was used to examine the group effect where left and right thalamic variables served as the within factor. There was no group x hemisphere interaction for any of the thalamic variables.

6.7.3 Fractional Anisotropy

Diffusion values were computed for bilateral thalamus using the following formula for weighted average:

$$average = \frac{(lx\ X\ lv) + (rx\ X\ rv)}{lv + rv}$$

where *l* = left thalamus, *r* = right thalamus, *v* = thalamic volume and *x* = the diffusion variable of interest e.g.: FA or MD. Fractional anisotropy was skewed in both hemispheres (Left: SW-W = 0.95, $p = 0.02$; Right: 0.95, $p = 0.02$) but this was only slight and there were no other assumption violations so analysis continued as normal. Fractional anisotropy measures did not reflect cognition. Adding demographic (*Figure 6-6*) and clinical [$F(2,84) = 0.25$, $p = 0.78$] covariates did not change results.

6.7.4 Mean Diffusivity

Mean diffusivity values were computed using the weighted average formula and although the mean values for the left hemisphere (SW-W = 0.91, $p < 0.01$) were slightly skewed analysis continued as normal as all other assumptions held. Mean diffusivity was the integrity measure that was most sensitive to cognition. Relative to control subjects, cellular disruption was evident in PD-MCI ($p = 0.02$) with a further increase in PD-D relative to PD-MCI ($p = 0.04$). Mean values were higher in both the PD-MCI ($p = 0.02$) and the PD-D ($p < 0.001$) group, indicating the result was not only due to PD motor symptoms. In contrast to volume and fractional anisotropy results, mean diffusivity was the only thalamic variable which did not have an effect of hemisphere [$F(1,113) = 3.47$, $p = 0.06$]. The right hemisphere was slightly more affected in the control group, but this gap closed as cognition worsened and did not result in a group by hemisphere interaction [$F(3,113) = 0.08$, $p = 0.97$]. The addition of demographic (*Figure 6-6)* and clinical covariates did not change the results, the PD-MCI group continued to exhibit higher MD values than PD-N ($p = 0.02$) with a further increase in PD-D relative to PD-MCI ($p = 0.03$).

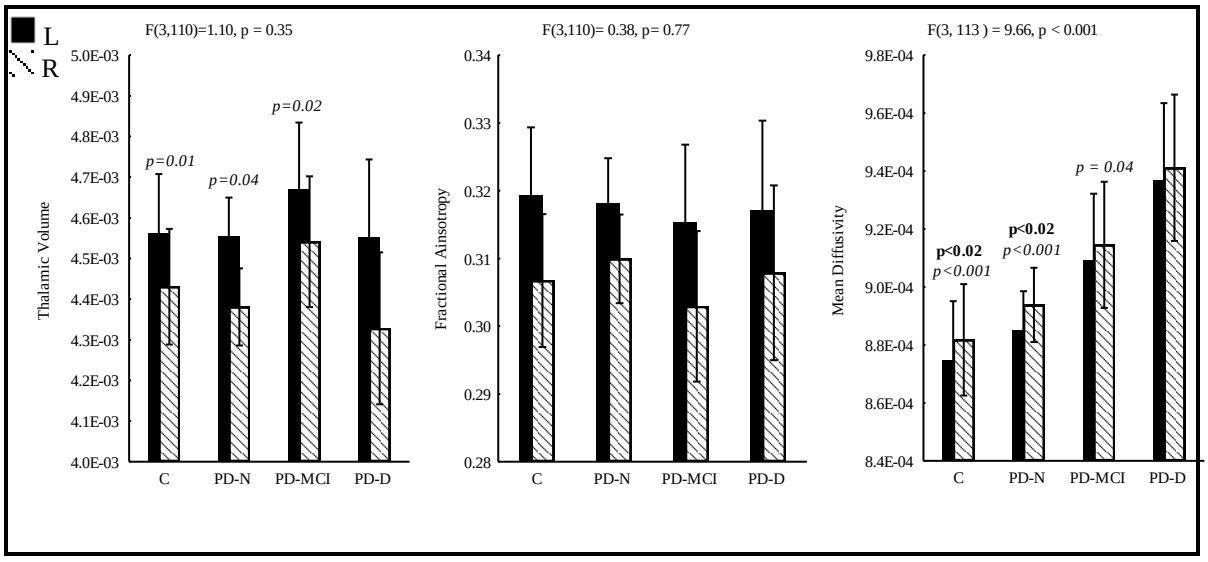


Figure 6-6: Volume, and diffusion measures of thalamic integrity
Thalamic volume and integrity differences between groups. **L:** = Left; **R:** = Right, lines represent standard error bars. Mean diffusivity was the most sensitive measure to cognitive dysfunction and showed a progressive increase in value while thalamic volume was only significant lower in the dementia group.

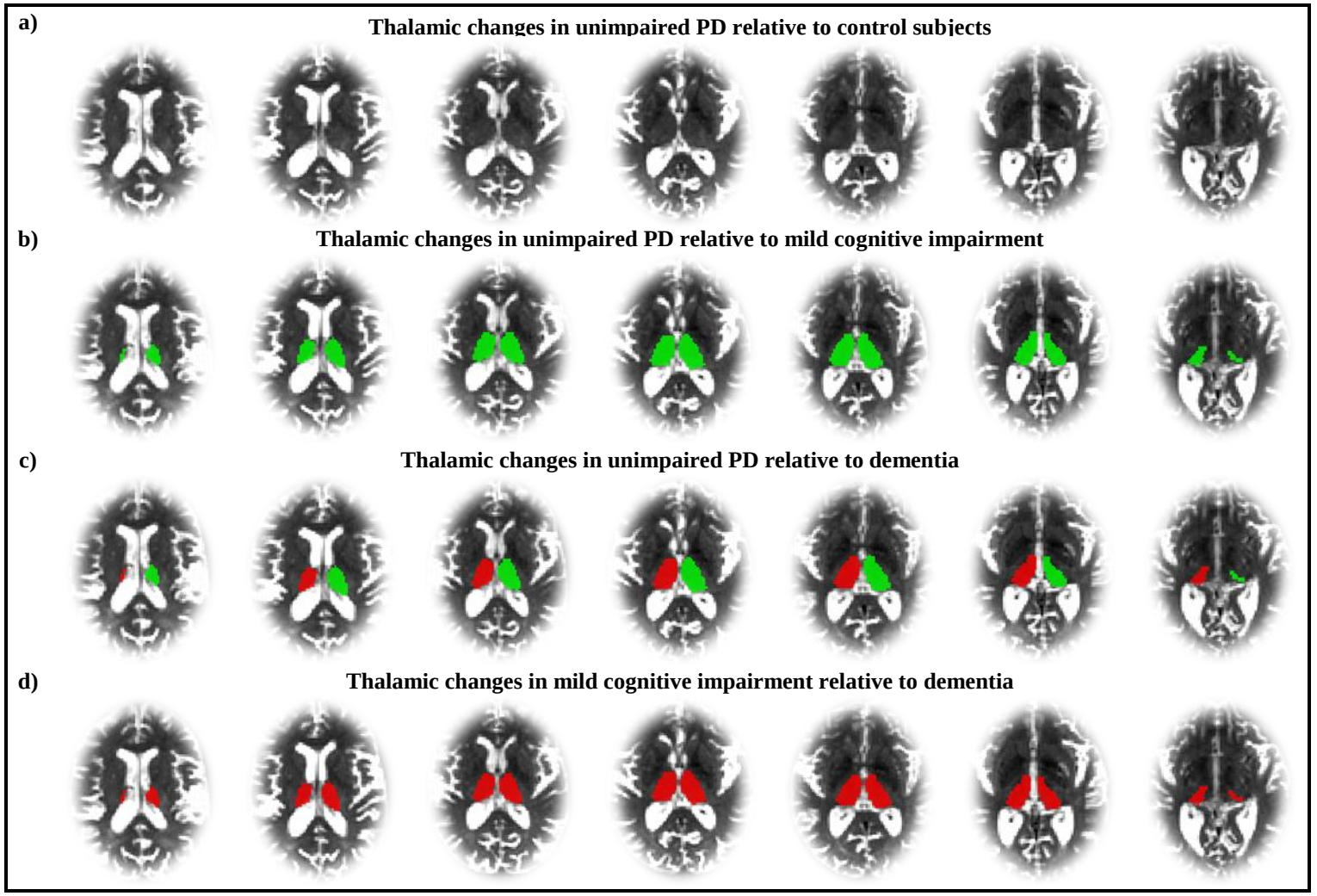


Figure 6-7: Thalamic changes in PD patients with cognitive impairment
Horizontal sections are displayed using radiological convention, the left side of the image corresponds to the right side of the brain. Slices are presented from the most dorsal slice to the most ventral slice where the thalamus is visible. Rows **a)** – **d)** show thalamic mean diffusivity (green) and volumetric (red) changes after accounting for co-variates. There were no instances where there was significant volume loss without a significant increase in MD. Image is 4D image with MNI space co-ordinates Z: 30-24

6.7.5 The relationship between the thalamus and cognition

6.7.5.1 Correlation Results

In the first instance simple Pearson correlations were used to determine if the thalamus had an association with cognition. In the case of a relationship the association was then further examined using regression modeling. For each correlation the bilateral thalamus variables (volume, FA and MD) were independently examined for their relationship with each cognitive domain (attention, executive function, learning and memory, visuospatial/visuoperception and aggregate global score). Correlations were conducted separately for the control and patient groups following the recommendations of Little, et al., (2010) in order to counteract the possibility of a false association by virtue of the patients consistently exhibiting a greater variance in cognitive scores. The assumptions of normality, of a linear relationship between the two variables, of minimum outliers and homoscedasticity – where the variance along the line of best fit remained similar, held for all variables.

<u>In healthy control subjects</u>

In the healthy control subjects there was no association between any thalamic measures with any areas of cognition.

<u>In Parkinson's disease</u>

The same correlation matrices were applied to the patient group. Volumetric measures of the thalamus yielded a relationship with attention ($r = 0.21$, $p = 0.05$) and aggregate global Z score ($r = 0.21$, $p = 0.05$). Not surprisingly, there was no association between the thalamus and cognition when FA was used as the integrity measure. Conversely, MD showed an association with all cognitive domains: learning and memory, ($r = -0.40$, $p < 0.001$); executive function, ($r = -0.36$, $p < 0.001$); attention ($r = -0.37$, $p < 0.001$); visuospatial/perception ($r = -0.36$, $p < 0.001$) and global Z score, ($r = -0.41$, $p < 0.001$).

6.7.6 The influence of covariates

For those domains that had a significant correlation with the thalamus, the relationship between thalamic structure, integrity and cognition was then examined using multiple regression to determine the thalamic influence after accounting for covariates. Each

cognitive domain was examined using a separate model where that variable was entered as the dependent variable and either volume or MD was entered as the independent variable along with the covariates age, education, depression, and disease duration.

The assumption of normality in arrays held, each group was normally distributed around the criterion variable (predicted score – observed score). The residuals of each criterion variable were also normally distributed. The assumption of multi-colinearity did not hold for the UPDRS III and disease duration variables ($r = 0.44$, $p < 0.05$) or for left and right thalamus (Vol $r = 0.91$, $p < 0.001$; MD $r = 0.79$, $p < 0.01$) so the UPDRS III variable was excluded and only the average bilateral thalamus variables were used in the analysis. The assumption of linearity held in that the relationship between each criterion variable and each predictor variable was linear. Homogeneity of variance in arrays - that for any set of predictor variables the corresponding variance of criterion variable will be the same also held. The control participants were excluded for all models due to the fact there was no association between the thalamus and cognition in this group.

Multiple regression statistics are reported in *Table 6-5*. After accounting for the influence of covariates, there was no longer an association between thalamic volume and cognition. Mean diffusivity measures of the thalamus survived the inclusion of co-variates however and remained a predictor of all cognitive domains and the aggregate Z score. In every case the overall model accounted for a significant proportion of the variance in the respective cognitive domain with the thalamus contributing between 3-6% of the unique variance.

Table 6-5: Multiple Regression Analysis: Relationship between thalamic variables and cognitive domains adjusted for covariates (controls excluded)

			Proportion of variance accounted for (R^2)	
	Beta	p	Whole model	Thalamic measure
Volume				
Global Z score	-0.02	0.87	40%, p<0.001	-0.0002, p=0.87
Attention/wm/processing	-0.02	0.83	37%, p<0.001	-0.0003, p=0.83
Mean Diffusivity				
Global Z score	-0.25	>0.01	45%, p<0.001	5%, p<0.01
Attention/wm/processing speed	-0.19	0.05	40%, p<0.001	3%, p=0.05
Executive function	-0.19	0.04	47%, p<0.001	3%, p=0.04
Learning and memory	-0.27	>0.01	33%, p<0.001	6%, p<0.01
Visuospatial/perception	-0.25	0.02	31%, p<0.001	5%, p=0.02

Thalamic volume and mean diffusivity were entered in multiple regression analysis as predictors of cognitive domain scores along with the covariates age, education, depression, disease duration. The UPDRS III was excluded due to co-linearity with disease duration.

Thalamic MD values were then used to predict cognitive function using the following regression equation:

*Cognitive domain score = constant – (intercept * MD).*

These results are presented graphically in *Figure 6-8* and show a clear difference in group distribution. The PD-D patients (red), clustered around the lower ends of the graphs show high diffusivity and low cognitive function. This group also has the largest variance in scores. The control (blue) and PD-N (green) groups are clustered around the upper ends of the graphs, showing low diffusivity and high cognitive scores with the PD-N group showing higher variance than the controls. The PD-MCI (yellow) group represents an intermediate stage, exhibiting cognitive scores corresponding to a medium level of thalamic degeneration. The groups all show significant overlap, especially in regards to PD-N and PD-MCI, suggesting that they will not be easily distinguishable by thalamic MD alone.

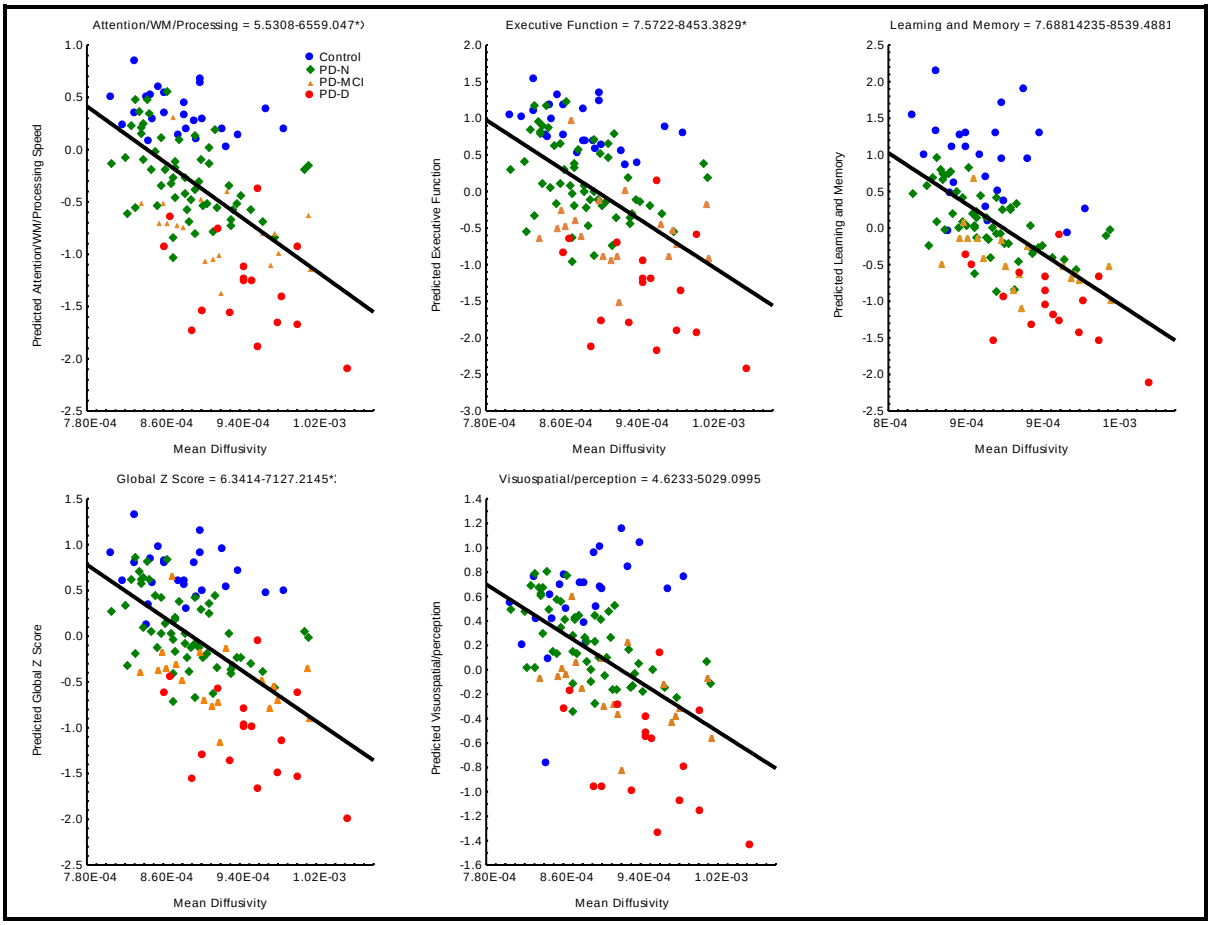


Figure 6-8: The association between thalamic mean diffusivity and cognition
Thalamic mean diffusivity is a unique predictor of each cognitive domain and the regression equation can be applied to show the predicted level of cognitive function for each individual.

6.7.7 Using thalamic measures to aid in group discrimination

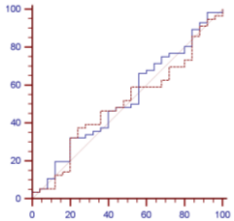
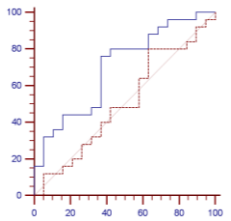
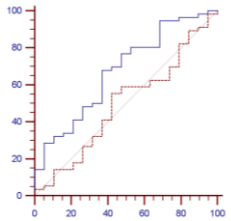
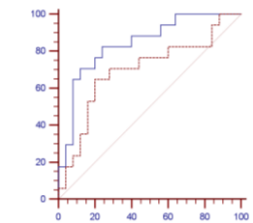
Receiver operating characteristics were conducted to determine the measure most sensitive to cognitive dysfunction in the PD groups. In each case, analyses were only conducted using MD and volume measures and FA was not included as there was no indication this measure of thalamic integrity had an effect on cognition in this sample. Optimal diagnostic values are reported in *Table 6-6* where specificity (the probability of true negatives) is >80% and positive predictive values (the probability of an accurate positive test) are >80% when available. The following pair-wise comparisons were examined: PD-D from control, PD-N and PD-MCI; PD-MCI from control and PD-N and, to determine the effect of Parkinson's disease alone PD-N from control. The assumption that all groups were normally distributed was upheld.

6.7.7.1 Prediction of the patient group

Thalamic volume was not sensitive to cognition in this sample, and generally did not result in successful identification of those groups who were exhibiting any level of cognitive impairment. The only exception was in regards to the PD-D group which showed a significant area under the curve (AUC) compared to the control and PD-N groups, enabling correct identification of dementia 65% and 53% of the time respectively.

Mean diffusivity, on the other hand was more sensitive to cognitive dysfunction, showing significant AUC statistics for all pairwise comparisons except between the control subjects and PD-N. Not surprisingly, the further removed the cognitive groups were from each other on the spectrum of cognitive dysfunction, the easier it was to differentiate between them. Identification of the dementia group was more successful when the control group, rather than the PD-N group was the comparison sample for example, although both were achieved with high success. Similarly, identification of PD-MCI was also easier when the comparison group was the control rather than PD-N group. Together, dementia was successfully identified approximately 80% of the time while PD-MCI was identified approximately 70% of the time.

Table 6-6: Separation of cognitive groups using thalamic integrity measures, RoC results

	Predicting PD	Predicting mild cognitive impairment		Predicting dementia	
	Control	C vs PD-MCI	PD-N vs PD-MCI	C vs PD-D	PD-N vs PD-D
Thalamic Volume					
AUC (95% CI)	0.50 ns (0.39-0.62)	0.5ns (0.35-0.66)	0.50ns (0.34-0.61)	0.70[c] (0.54-0.83)	0.70[b] (0.58-0.80)
Sensitivity	32.1	36.8	14.3	64.7	53.0
Specificity	80.0	80.0	84.2	80.0	80.4
Thalamic Mean diffusivity					
AUC (95% CI)	0.53ns (0.41-0.64)	0.70[b] (0.54-0.83)	0.68[c] (0.56-0.78)	0.84[a] (0.69-0.94)	0.82[a] (0.71-0.90)
Sensitivity	32.1	57.9	47.4	76.5	70.6
Specificity	80.0	80.0	80.4	80.0	84.0
					

AUC: area under the receiver operating curve (chance = 0.5; perfect separation = 1.0). Superscript numbers indicate level at which the comparison is significant, a: $p < 0.001$; b: $p < 0.01$; c: $p < 0.05$.

6.8 Discussion

6.8.1 Summary of the results

The study in this chapter used a cross-sectional design to investigate the thalamus as a potential biomarker for cognitive impairment in Parkinson's disease. We expected that thalamic integrity and volume would be reflective of cognitive dysfunction in PD. The thalamus was identified *a priori* as an area for investigation due to its many cortical connections with areas that are known to be implicated in cognitive dysfunction in PD (Taber, et al., 2004). Volume, fractional anisotropy and mean diffusivity of the whole thalamus were all examined for a relationship with cognition from structural images in native subject space. Thalamic volume had a relationship with various cognitive domains but this did not hold once co-variates were accounted for. Fractional anisotropy did not have any relationship with cognition. Mean diffusivity on the other hand was significantly associated with multiple facets of cognition and resulted in an overall group difference, an independent relationship between thalamus and cognition and the ability to differentiate between the similar cognitive groups within Parkinson's disease.

6.8.2 Thalamic volume reduction

In terms of thalamic volume, the hypothesis was supported but significant volume reduction was only evident in the dementia group. In the PD-MCI group, volume loss was only at trend level. In the PD group who had no cognitive impairment there were no volume changes compared to control subjects. Together, results indicate that thalamic volume is related to severe cognitive dysfunction, is not sensitive to mild cognitive decline and not reflective of other Parkinson symptoms. Because thalamic atrophy has been shown to decline with age (Sullivan, Rosenbloom, Serventi, & Pfefferbaum, 2004) our results are reported after accounting for age effects in ANCOVA models and after each PD patient in our group was age matched to one of our healthy control participants.

The thalamic atrophy in the PD-D group that was identified in this study has not previously been investigated in native subject space. Thalamic atrophy in PD-D has previously been identified in standardised space (Burton, et al., 2004; Summerfield, et al., 2005) however and supports our findings, showing a significant reduction relative to control subjects. The comparison between our findings and those of others in standard space will be more thoroughly explored in the subsequent chapter (*Section 7.3.2*). The

ventricles that surround the thalamus have previously shown significant dilation (Camicioli, et al., 2011) in PD-D, identified in native subject space. This previous study supports our findings as an increase in ventricles is thought to relate to the atrophy of the surrounding structures, including the thalamus (Gaser, et al., 2004).

In regards to the trend level atrophy in the PD-MCI group, only one study (Dalaker, et al., 2011) has previously examined the thalamus in PD-MCI and report's findings similar to our own, there was no atrophy in the thalamus of this group compared to control subjects. The criteria used to categorise PD-MCI in Dalaker and colleagues sample was less stringent than our own and only required patients to exhibit impairment in one cognitive domain rather than two (Aarsland, et al., 2010). It is therefore, somewhat surprising that our own sample who is exhibiting impairment closer to PD-D than PD-N shows no thalamic atrophy.

More research has been conducted on PD patients without dementia. Generally, only basic criteria has been used to exclude dementia in these patients so sub-threshold cognitive impairment is still possible. Nevertheless, three previous research groups (McKeown, et al., 2008; Messina, et al., 2011; Peran, et al., 2010) support our findings of no thalamic atrophy in PD compared to normal control subjects and two (Lee, et al.; Lisanby, et al., 1993) contradict our findings, showing significant levels of atrophy in the thalamus. The most obvious reason for the contrast in findings is that cognitive dysfunction was only examined using the MMSE in the Lee, et al., (2011a) sample and not at all in the Lisanby, et al., (1993) sample. Thus, it is highly likely that instead of being PD patients with no disenable cognitive impairment; the patients were in fact exhibiting significant levels of impairment which was reflected in the degree of thalamic atrophy. Given the insensitivity of the MMSE, and that the Lee et al., PD patients had a score of 27.4 (SD 1.8) cognitive impairment not being detected is a likely scenario.

We have used a large sample of PD patients, extensive neuropsychological testing and robust statistical analysis and although our findings are in contrast to some previous studies, they are not in contrast to those studies that have also used more extensive neuropsychological examination and statistical analysis. We have also shown that level of thalamic atrophy was useful in discriminating between PD-D and all other PD groups, as reflected in our RoC analysis. Thalamic volume could effectively discriminate between the dementia group and: the control; PD-N and PD-MCI groups. It is especially relevant that the subtle difference between PD-MCI and PD-D, but not the difference between PD-N and PD-MCI was able to be detected in our sample. Due to the controversy currently

surrounding the diagnosis of PD-MCI (Dalrymple-Alford, et al., 2011) we propose that thalamic volume could effectively be employed as a tool to aid in discrimination between PD-MCI and PD-D.

6.8.3 Thalamic cellular disruption

Mean diffusivity changes are assumed to reflect increases in the extracellular space as a result of cellular degeneration and fractional anisotropy measures are assumed to relate to the disruption in white matter fiber tracts (Kantarci, et al., 2001). We expected significant cellular alterations in our PD groups without dementia as cellular disruptions such as these occur prior to gross levels of atrophy (Peran, et al., 2010). Our results show that, although thalamic volume reduction was not evident in our PD-MCI group, mean diffusivity of the thalamus was significantly altered relative to the PD-N group. This only partly supports our hypothesis as we also expected fractional anisotropy measures to show significant alterations but FA remained at the same levels across all groups.

The significant disruption in diffusion in the absence of volumetric changes supports the findings of Peran, et al., (2010) who is the only other group to have examined both the micro and macro structure of the thalamus in PD. In their sample, microstructural alteration of the thalamus was identified using both FA and MD measures and occurred in the absence of any greater volume changes.

6.8.3.1 Fractional anisotropy differences

Fractional anisotropy values of the thalamus did not support the hypothesis, there was no group effect nor a trend for change in any of the PD groups compared to controls. This is in contrast to Peran, et al., (2009) who reported significant FA reduction in the thalamus of PD relative to controls. Significantly lower FA has also been reported in Alzheimer's disease - a disorder characterised by its cognitive dysfunction, (Canu, et al., 2010). Unfortunately neuropsychological testing was not conducted in the Canu, et al., (2010) sample but given that AD had been diagnosed, patients were probably exhibiting similar levels of cognitive impairment seen in our own PD-D group. The most likely reason for the discrepancy between their findings and our own is that the profile of cognitive impairment in PD is different to that of the more common dementia of the Alzheimer's type. Given that both disorders are neurological in origin, the different manifestation of symptoms could be due to pathology in different cortical regions; differentially affecting the thalamus. In PD-D for example, the overall cognitive profile is of severely reduced

visuospatial function followed by impairment in memory and then attention and executive function (Emre, 2010). Visuospatial deficits are particularly impaired in PD-D compared to AD as patients have difficulty with the visual perception of objects and visual discrimination between objects (Mosimann, et al., 2004). Visuospatial function is not typically attributed to the thalamus and is more the domain of the posterior cingulate and medial temporal – frontal lobe circuitry (Kravitz, Saleem, Baker, & Mishkin, 2011).

The biggest contrast between the two disorders is the level of memory dysfunction, the thalamus has strong connections with the limbic system which mediates memory processes (Aggleton & Brown, 1999) and this could lead to a significant relationship between the thalamus and cognitive dysfunction in Alzheimer's but not Parkinson's disease. Memory deficits are the hallmark of AD but attention and executive function are most affected in PD-D (Bronnick, et al., 2007) and a memory deficit is not specifically required for diagnosis of PD-D (Emre, et al., 2007). Some patients do experience significant trouble with working memory (Emre, 2003) and mainly struggle with keeping information 'in mind.' For example, when asked to repeat back a sequence of numbers – either in the order given to them, or in reverse order, patients will not be able to remember which numbers, beyond a few that were presented to them (Woods & Troster, 2003b).

An extensive meta-analysis undertaken by Emre, (2010) shows that verbal memory is deficient in PD-D compared to control subjects, but it still remains better than AD patients. Patients will generally experience episodic memory deficits where the memory content of past events is not able to be consciously accessed (Baddeley, 2002). It is interesting that in a phenomenon specific only to PD, patients are able to recognise stimuli when it is presented to them, but may not be able to verbally recall the information on request (Dubois & Pillon, 1997). In the California Verbal Learning Test, for example, a series of words are presented to a patient and he is asked to recall these at a later time. For patients with dementia the recall is very difficult and they typically cannot provide more than a few words. Conversely, when these same words are presented visually and the patient is asked to confirm whether they were part of the original test sample, performance rate is significantly higher (Breen, 1993).

Another possibility is that the white matter that runs through the thalamus was not large enough to reflect FA changes in our sample. This has been demonstrated in Parkinson's disease before when the tracts of the basal ganglia were examined (Nilsson, et al., 2007). The authors found that although there were increased MD and decreased FA values in the region of the nigrostratial tract it was not possible to track the white matter

tract through this region as they were only of a minor magnitude. The integrity of the thlamic-cortico tracts may be better measured using tractography as this has been successfully applied to examine basal ganglia-thalamocortical circuit connectivity in PD before (Hamasaki, Yamada, Hirai, & Kuratsu, 2010). After stimulation of sub-thalamic nucleus, those patients who had greater white matter volumes showed greater improvement in symptoms, thought to be due to the better connectivity in the thalamocortical circuit. These results indicate that although the tracts within the thalamus of our PD patients may not have been detected using diffusion imaging in this instance, they could potentially be large enough to be examined successfully using a tractography approach – a hypothesis which is examined in *Chapter 9.*

6.8.3.2 Mean Diffusivity Differences

Mean diffusivity measures of the thalamus did support the hypothesis, the groups with higher cognitive impairment have higher MD values. In our sample, the dementia group shows the most cellular alterations, with thalamic MD values significantly higher than both the control and unimpaired group. After excluding the control participants in order to control for clinical co-variates, MD values are higher than both the unimpaired and MCI group. To the best of our knowledge this is the first study to find increased diffusivity in the thalamus of PD patients which has a relationship with cognition and also show significant comparisons between the dementia group and other PD groups without dementia. This indicates that thalamic changes cannot be solely due to the motor symptoms of PD.

Diffusion values of the thalamus have been examined in PD before and confirm our results. Nicoletti et al., (2006) reports trend level increases in PD compared to control subjects who did not meet criteria for dementia. Peran (2010) also reports higher diffusivity in PD without dementia compared to control subjects, at a significant level. Increased diffusivity in these samples could be reflective of cognitive dysfunction as there is no increases in diffusivity between patients with PD and those with other parkinsonism disorders of a highly motor phenotype. In the Rizzo, et al., (2008) sample for example PD, PSP and CBDS patients were all examined using DTI. Diffusivity differences were only approaching significance between control subjects and all patient groups, indicating the motor dysfunction and disease duration evident in these groups was not reflected in thalamic disruption. Cognition was not examined but all subjects Hoehn and Yahr and UPDRS scores reflect examination in the off state, indicating motor dysfunction was at a

level worse than normally reported in motor disorders and there were still no cellular changes in thalamus.

We have also shown that thalamic diffusivity is a reliable biomarker that can discriminate between the different cognitive subtypes of Parkinson's disease. Mean diffusivity values show a significant area under the curve in RoC analysis for comparisons between the unimpaired and MCI and dementia groups. Raw mean diffusivity values cannot distinguish between the similar PD-MCI and PD-D groups however. Interestingly, after adjusting for co-variates including age, disease duration, depression and motor score the adjusted MD is able to distinguish between these two groups.

Other attempts have been made at identifying biomarkers for Parkinson's disease but, as yet there is currently no well established biomarker for the distinct cognitive levels within PD (Breen, Michell, & Barker, 2011). Because deficits in executive function, visuospatial function and memory are present even in the very early stages and indicate higher chance of a later progression to dementia (Janvin, et al., 2006), it is important that these symptoms are identified as early as possible.

Other attempts have been made to investigate the earliest biomarkers of cognitive dysfunction in PD but are applied with limited success. Amyloid B concentrations in newly diagnosed PD patients, for example, had a significant linear relationship with memory, but are not useful for examination with visuospatial and executive dysfunction (Alves, et al., 2010). The level of cortical plaques can be directly measured by looking at the amount of amyloid beta in the cerebral spinal fluid as there is an inverse relationship between B-Amyloid and plaques (Strozyk, Blennow, White, & Launer, 2003). Another study (Compta, et al., 2009) showed that amyloid B levels in CSF are higher in control subjects, intermediate in PD patients without dementia and lowest in PD-D patients, and could prove to be an early marker for cognitive dysfunction. There is a strong association between this measure and semantic fluency – an executive function measure, known to be impaired in the early stages. In this study the PDND patients had lower scores in memory, executive function and visuospatial functioning than controls, indicating that most individuals were exhibiting some degree of mild cognitive impairment but as this was not specifically diagnosed according to any criteria it is difficult to establish to what degree cognition was impaired in this group.

The sensitivity of MD to the different levels of cognitive dysfunction in our PD groups has also been reported in other neurodegenerative studies. In Alzheimer's disease for example, when cognitive impairment is advanced the thalamus shows both structural

degeneration and a significant disruption in diffusivity (Zarei, et al., 2009) but in pre Alzheimer's mild cognitive impairment there is no structural difference in the volume of the thalamus between AD-MCI and AD, although there is a significant increase in diffusivity between the two groups (Cherubini, et al., 2010).

In contrast to the majority of research that has examined PD-MCI we have diagnosed MCI within the PD group according to criteria that has proven to be valid when compared against other criteria (Dalrymple-Alford, et al., 2011; Litvan, et al., 2012). This makes diffusivity a stronger biomarker in PD as varying levels of cognitive impairment, not just dementia, can be identified.

6.8.4 Thalamic influence on cognition

The relationship between the above level of thalamic disruption is inferred to be reflective of cognitive dysfunction due to the higher levels of cognitive impairment evident in those groups who exhibit greater thalamic disruption. Robust statistical analyses further examined the relationship in order to control for extraneous variables. Mean diffusivity measures of the thalamus had the strongest relationship with cognitive dysfunction in this cohort. For the first time, this research found a strong relationship between MD and multiple cognitive domains. It was the only measure of thalamic integrity that was an independent predictor of: attention/working memory/processing speed; executive function; visuospatial/visuoperception and learning and memory as well as global Z score. After adjusting for demographic and clinical co-variates MD still had a strong independent relationship with the cognitive variables and could be practical in estimating the level of thalamic integrity disruption once their global Z score is known.

Thalamic diffusivity has clearly shown a relationship with cognitive domains in multiple disorders (de Jong, et al., 2008; Houtchens, et al., 2007) but no other disorders have been examined with such extensive neuropsychological testing, making this research the first to show thalamic diffusion measures have a strong independent relationship with multiple areas of cognition as measured by several different tests.

In other neurodegenerative disorders, the results between thalamic diffusion values and cognition are varied and difficult to examine due to the different methods used to quantify cognitive function. Thalamic MD has shown an independent relationship with cognition in Schizophrenia (Spoletini, et al., 2009). There was a clear disease effect, thalamic diffusivity was higher in patients compared to controls and there was a correlation between the right thalamus of the schizophrenic group and working memory. In temporal

lobe epilepsy (Wang, et al., 2010) there was a strong relationship between diffusion values of the right thalamus and a category fluency task (a measure of executive function). In Multiple Sclerosis, mean diffusivity of the thalamus is increased in patients compared to controls, and has a strong relationship with the paced auditory serial addition task with is a measure of information processing and attention (Tovar-Moll, et al., 2009). In Alzheimer's disease (Canu, et al., 2010), patients were diagnosed as having moderate to severe AD and showed increased diffusion in multiple subcortical regions, including in the thalamus bilaterally. When grey and white matter volume loss were included as co-variates, only the effect in the right thalamus remained significant.

From results of other disease samples it is unlikely that FA is related to cognition, a result that also supports our findings. In the temporal lobe epilepsy example, reduced FA was seen in bilateral thalamus of patients compared to controls. FA showed a direct relationship with epilepsy specific characteristics such as age of seizure onset and duration of epilepsy but did not show any relationship with cognitive measures (Wang, et al., 2010).

6.9 Summary

Here, we have applied comprehensive cognitive testing to a large sample of Parkinson's disease patients of varying cognitive state. We have used advanced medical imaging and analysis techniques to examine the differences between subject groups and report results that have taken the motor confounds of Parkinson's disease into account. We report that the microstructural integrity of the thalamus is compromised in Parkinson's disease, even when thalamic volume is maintained. This subtle abnormality shows a strong relationship with multiple areas of cognition and can be used to discriminate between the cognitive subtypes of PD. The practical applications of these results are widespread. Cognitive level of a patient can already be identified within Parkinson's disease using neuropsychological tests and accepted criteria for diagnosis. Although the criteria for PD-D is widely used, controversy still surrounds the PD-MCI criteria, and in some cases a patient is identified as unimpaired when other criteria would diagnose them with MCI (Dalrymple-Alford, et al., 2011). An incorrect diagnosis is distressing for patient and family and we hope, by exhibiting that the thalamus can be used as a biomarker for cognitive impairment we have gone some way in aiding the detection of MCI in PD.

Chapter 7. Study Two: THE THALAMUS IN STANDARD SPACE

7.1 Objectives

The objective of this chapter was to compare our findings of thalamic atrophy and cellular disruption, identified in native space in the previous chapter (*Chapter 6*), to standard space analysis of the same groups. As the thalamus has been examined more frequently in standard, rather than native space, a secondary objective was to compare previous findings with our findings. Previous studies have typically applied whole brain analysis instead of region of the region of interest approach applied in our study so comparison between these two methodologies will also explored. Similar to the analyses conducted in the previous chapter, the relationship between thalamic integrity and cognitive function in Parkinson's disease will be examined and compared with that of the healthy control group. This chapter begins with a literature review which covers brain changes that have already been identified in Parkinson's disease using VBM. Again, the literature review is not restricted to changes only within the thalamus and will also address wider brain changes due to the influence the thalamus has on the wider neocortex.

7.2 Voxel Based Morphometry

Voxel based morphometry (VBM) is an automated imaging method which allows for comparison of all image voxels within any part of the brain. The whole brain can be examined without arbitrary definition of regions of interest or reliance on user consistency (Burton, et al., 2004) and without the requirement of a prior hypothesis about the areas of interest (Ashburner & Friston, 2000). Essentially, the VBM process involves segmenting individual MR images into grey, white and cerebral spinal fluid. The integrity of the grey and white matter tissue reflects the integrity of underlying neurons and synapses. Grey matter, for example is made up of neuronal cell bodies, dendrites, axon terminals and glial cells - although it is impossible to tell to what degree each component is represented. White matter volumes mainly consist of the mylenated axons which connect neuronal bodies (Kanai & Rees, 2011). The tissue to be studied – typically only the grey and white matter, is transformed to standardised space and each groups' images are averaged to a template. The voxel within each image is then able to be compared and any areas of difference between groups is able to be identified (Good, et al., 2001). VBM is

advantageous in that the whole brain volume can be extracted from other non brain matter such as the skull or sinus tissue and grey and white matter volumes can also be extracted and compared independently (Bozzali, Cercignani, & Caltagirone, 2008).

7.3 VBM in Parkinson's disease

In PD, there are grey matter changes in both cortical (Camicioli, et al., 2009; Cordato, Duggins, Halliday, Morris, & Pantelis, 2005; Pereira, et al., 2009) and subcortical (Brenneis, et al., 2003) areas. In PD patients who also have dementia, grey matter density occurs to a greater degree and in wider regions than those that are implicated in PD (Lee, et al., Ibarretxe-Bilbao, Tolosa, Junque, & Marti, 2009; 2010). In the thalamus, grey matter density is only reduced in PD dementia patients, (Beyer, Larsen, et al., 2007; Burton, et al., 2004; Nagano-Saito, et al., 2005b; Summerfield, et al., 2005), showing no change in PD without dementia (Burton, et al., 2004; Nagano-Saito, et al., 2005b; Summerfield, et al., 2005). The protocols applied to VBM methodology can vary widely, add to this the inevitable variation between patient samples and controversy surrounding the details of grey matter loss in PD remains. Some studies report no change in PD (Price, et al., 2004) or PD-D (Sanchez-Castaneda, et al., 2009) compared to healthy control subjects.

7.3.1 Grey Matter Changes in Parkinson's disease without dementia

In PD patients without dementia grey matter decreases are evident in both cortical and subcortical regions. The degree and distribution of integrity changes described are widely variable however, mostly due to the criteria that is used in the diagnosis of dementia. In some cases the DSM-IV criteria (2000) is applied, but generally neuropsychological testing is limited.

Only the caudate nucleus (Brenneis, et al., 2003) and the area surrounding the left intraparietal sulcus (Cordato, et al., 2005) have been implicated in PD patients without dementia, showing significant volume loss compared to healthy control subjects.

When more advanced neuropsychological testing has been applied, the relationship between grey matter changes and cognition is further supported. Camicioli, et al., (2009) used several neuropsychological tests (DRS, CDR and MMSE) to examine cognition and exclude dementia in an older PD group. Grey matter loss was only evident in the bilateral cerebellum, an area that is normally involved in motor function but also plays a role in some cognitive functions (Gordon, 2007). Despite no changes in the Camicioli, et al., study, the integrity of several structures including the temporal gyrus, temporal lobe and

putamen did have a significant correlation with the long delay free recall component of the California Verbal Learning Test. This structure-function relationship was independent of any age effects. The caudate nucleus and the temporal lobe also correlated with a composite measure of executive function - derived from several tests including the Stroop, the Trail Making Test and the Digit Ordering Test, suggesting that in this sample at least, slight grey matter changes in some areas have a relationship with cognition in PD.

Along with memory and executive function, visuoperception also has a relationship with grey matter deficits in both cortical and subcortical structures in PD. In a sample of PD patients without dementia (Pereira, et al., 2009), for example, visuoperceptual function was significantly worse in the PD patients compared to control subjects. Visuoperception ability in this instance was an aggregate of tests of facial recognition, recognition memory and visual discrimination. Grey matter integrity of the parietal, occipital and frontal regions, as well as the fusiform gyrus in the temporal lobe had a significant correlation with the facial recognition task. Subcortically, the parahippocampus was also correlated with the facial recognition task.

In contrast to the above results, Price, et al., (2004) reported no areas of grey matter changes between PD and control subjects after examining the whole brain using VBM. The protocol that was applied to both the Camicioli, et al., and Price, et al., studies originated from the same group and has been validated in some large samples (Ashburner & Friston, 2000; Good, et al., 2001) indicating this discrepency between results is likley due to variation in a different aspect of the methodology that was applied in each study.

7.3.2 Grey Matter Changes in Parkinson's disease with dementia

In PD patients with dementia there are multiple cortical and subcortical regions of the brain that show distinct patterns of grey matter loss. Compared to a control sample, grey matter loss has been identified in the bilateral prefrontal, temporal, occipital and right parietal areas of PD-D patients (Lee, et al., 2010). Subcortically, grey matter loss is primarily evident in areas that are known targets of Lewy body inclusions (Braak, et al., 2003) including the hippocampus and cingulate gyrus (Ibarretxe-Bilbao, et al., 2009).

The location and degree of grey matter loss in PD still remains controversial however, even in PD dementia. Neither of the above studies adjusted for covariates, indicating that the grey matter loss in this instance may be due to effects of age or disease duration, rather than dementia. When years of education and the severity and duration of PD has been controlled for in PD-D (Sanchez-Castaneda, et al., 2009), no areas of grey

matter change were identified. In this same sample, the left parietal cortex and right occipital lobe did show reduced grey matter prior to adjustment for covariates, suggesting that patient characteristics other than their cognitive function are influencing results. In the Sanchez-Castaneda, et al., (2009) sample, although cognition was examined there was no relationship between grey matter density and measures of attention, memory or construction abilities, further supporting the idea of grey matter degeneration being influenced by factors other than cognitive dysfunction.

7.3.3 White matter changes in Parkinson's disease and Parkinson's disease with dementia

Some PD samples also show distinct patterns of integrity reduction in white matter which appears to occur in the absence of grey matter changes. In very early stage PD patients (disease duration = 3 years) for example, there was a significant reduction in white matter of the right fusiform gyrus and temporal gyrus compared to control subjects (Martin, Wieler, Gee, & Camicioli, 2009). Although these areas are commonly implicated later in PD (Beyer & Aarsland, 2008), at the time of examination patients were not showing any atrophy. Similarly, in another group of early PD patients (Disease duration = 6.33 years) without dementia there was a small cluster of white matter reduction in the brainstem of the PD group compared to the control group. This result was independent of age, disease duration and severity and also occurred in the absence of grey matter changes (Jubault, et al., 2009).

Increases in white matter density have also been observed in early-mid stage (disease duration 5.5 years) PD patients without dementia (Focke, et al., 2011). There was a small cluster of increased white matter volume in the right superior longitudinal fasciculus but this did not remain significant after whole brain correction. White matter changes may occur prior to grey matter changes in the early stages of PD, but when dementia has developed grey matter, rather than white matter changes predominate. In the Lee, et al., (2010) sample mentioned earlier, for example, areas of white matter reduction are less widespread than grey matter reduction and localised to the left temporal, occipital and prefrontal areas while grey matter reductions were evident in bilateral dorsolateral prefrontal, temporal, occipital, posterior cingular and right parietal cortical areas.

7.3.4 The progression from PD to PD-D as measured by VBM

The comparison between PD and PD-D and control subjects shows a distinct pattern of cortical and subcortical changes different to that of DLB (Lee, et al., 2010) or AD (Almeida, et al., 2003) indicating that it is pathology specific to PD that underlies these changes. Within PD, cortical changes are more evident in PD-D than in PD.

A recent cross sectional study (Nisho, et al., 2010) has shown that in PD patients with no cognitive impairment there is still large areas of change in the frontal, temporal and occipital cortices compared to the control group. In the PD-D group of this same study further grey matter decreases were evident in the same areas that were implicated in the PD group and also in the parietal cortex and cerebellum when compared with the PD-N group. All contrasts in this study were independent of movement impairment confounds (UPDRS-III) suggesting frontal, temporal and occipital grey matter reduction contributes to dementia in PD.

Some longitudinal studies have isolated regions most involved in the progression from PD to PD-D. Longitudinal studies follow patient groups for the duration of the disease and, as such, allow for greater interpretation of grey matter correlations with the cognitive symptoms of PD. Burton, et al., (2004) employed both cross-sectional and longitudinal methods to show the progression of grey mater changes. Compared to the control subjects, only the frontal lobes were reduced in PD-N. In PD-D, grey matter loss extended to the frontal, temporal, occipital and parietal lobes as well as subcortical structures within the limbic system. At one year follow up (Burton, McKeith, Burn, & O'Brien, 2005) in a small subset of the original cohort atrophy was significantly increased in these same areas in PD-D compared to both the PD and the control group. Total grey matter density was significantly associated with cognition, directly highlighting the influence of grey matter degeneration in the development of dementia.

The progressive nature of grey matter reduction in PD was also explored by Ramirez-Ruiz, et al., (2005). PD patients with and without dementia were initially examined and re-examined at two year follow up. In the PD patients without dementia, grey matter loss was primarily restricted to the subcortical limbic regions; the hypothalamus, nucleus accumbens and left hippocampus all showed some degree of grey matter reduction. In PD-D patients, grey matter loss was mainly in cortical regions. At follow up the limbic and wider temporal regions all showed further reduction in the PD patients. In the PD-D groups there was widespread grey matter loss in the temporal

regions. Cognitive function was measured using several neuropsychological tests, all of which concentrated on learning and memory. When co-varied against the degree of grey matter loss, the grey matter differences between pre and post MRI lost significance, supporting the idea that grey matter loss in PD is significantly related to cognitive decline.

7.3.5 Identifying grey matter changes in PD-MCI as a precursor to dementia

The degree of grey matter atrophy in PD also appears to be related to the time of dementia onset. Those with a shorter duration of PD prior to dementia (<8 years) show greater absolute and subcortical atrophy than those who develop dementia later in disease stage (Beyer & Aarsland, 2008). The first study to examine PD patients who also had MCI (Beyer, Janvin, et al., 2007) suggested that the degree of grey matter atrophy reflects the progression of cognitive impairment in PD. Beyer et al., (2007) showed that although there were no grey matter changes in PD patients who did not have MCI compared to healthy control subjects, in those that did have PD-MCI, the frontal gyrus and small areas within the bilateral temporal lobes were all significantly reduced. This result did not survive correction for multiple comparisons and was no longer significant when age was included as a co-variate in the ANCOVA analysis which renders the results questionable however. In contrast, the PD-D patients had areas of significantly reduced grey matter within the bilateral frontal, limbic, parietal and temporal lobes which survived corrections for the effect of age, suggesting changes are more robust in advanced stages of the disease.

Dalaker et al., (2010) also reports no change in grey matter density in any brain regions between PD-MCI and PD without MCI. Despite there being no obvious changes between groups there were some significant correlations between the association areas of the cortex within the frontal and temporal gyrus and reduced performance on a composite measure of attention/executive function. These were lost once the co-variates age, motor severity (UPDRS III) and intracranial volume were included in a regression analysis however.

The progressive nature of cortical changes in PD was also examined by Song and colleagues (2011). This research included comparisons between PD-MCI and PD-D but did not include a control group for comparison. Their results largely confirm that of the Beyer (2007) study with the exception that all grey matter differences remained significant after the inclusion of age, education, total GM volume and disease duration in ANCOVA. Some caution must still be applied when interpreting these results as no correction was made for multiple comparisons. Results showed that grey matter atrophy increased with

cognitive dysfunction, the PD-MCI group had decreased grey matter in the right frontal area compared to the PD-N group. In the PD-D group there were areas of decreased grey matter in bilateral temporal and left prefrontal areas compared to the PD-MCI group. The authors conclude that the location and degree of GM atrophy of PD-MCI appears to represent an intermediate stage between PD-N and PD-D, implying that PD-MCI is the initial stages of PD-D and this is reflected in cortical atrophy levels.

Recent work from our own lab Melzer et al., (2011b) was the first to fully investigate the progressive nature of PD, including comparisons between PD-N, PD-MCI, PD-D and a healthy control group in a cross-sectional design. The sample included 104 PD patients and 39 control participants matched for age, sex and education level, making it the largest PD sample of this nature to date. There was no grey matter atrophy evident in the PD-N group compared to controls, but density loss was clearly evident throughout the PD group as cognitive impairment worsened. In the PD-MCI patients, there was only moderate loss in temporal and frontal areas in comparison to control subjects. In PD-D there was more extensive loss in these same regions and other regions such as the prefrontal regions and, subcortically in the parahippocampus and the caudate nucleus.

As the cortical changes associated with cognitive dysfunction were a primary focus here, the PD-MCI and PD-D groups were further compared against the PD-N group and the UPDRS III score added as a covariate to control for any grey matter changes that may have mainly been influenced by the movement, rather than cognitive dysfunction within the PD groups. The intermediate nature of the MCI stage is immediately evident within these comparisons. Between PD-MCI and PD-N, grey matter reductions are slight compared to the loss that is evident in the PD-D group when compared to PD-N. There is also no further loss between the PD-MCI and PD-D group, indicating the grey matter changes detected in PD-MCI are subtle reductions and that only select cortical regions need to be affected before cognitive impairment is evident. There were no areas of change between PD-N and the control group, highlighting that the integrity changes throughout this cohort cannot be attributed to the motor dysfunction of PD alone.

Extensive neuropsychological testing was conducted in the Melzer, et al., (2011b) study – the same testing has been applied to a different sample in the course of this research (see *Chapter 5*). Regression analysis using an amalgamation of these neuropsychological measures showed a relationship between global cognitive score and areas of the: posterior cingulate cortex, parietal, occipital, frontal and temporal cortices as well as temporal subcortical regions including the hippocampus and medial thalamus. This

relationship persists after correcting for multiple comparisons and is unlikely to be due solely to motor deficits as there is only a small area of the frontal gyrus that had a relationship with UPDRS III score when cognitive score was co-varied for.

7.3.6 VBM and the thalamus

Grey matter loss in the thalamus has only been identified in PD-D. There is a significant bilateral (Burton, et al., 2004) and left (Summerfield, et al., 2005) grey matter reduction in PD-D compared to control subjects. Although both these studies also included a PD group without dementia for comparison, there were no significant grey matter changes between PD and PD-D.

A further research group (Nagano-Saito, et al., 2005a) and a later study by Beyer et al., (2007) has implicated thalamic degeneration in the cognitive, rather than motor symptoms of PD. The bilateral (Nagano-Saito, et al., 2005b) thalamus was reduced in a PD-D group compared to a PD group and, in the later study, the right pulvinar was also implicated in PD-D compared to a PDND. In this case, the PDND group also included some PD-MCI subjects which suggests the pulvinar is most implicated when cognitive impairment is advanced and is unlikely to show change when cognitive impairment is not evident (Beyer, Janvin, et al., 2007).

Not surprisingly, grey matter reduction in the thalamus has also been implicated in relation to movement dysfunction of PD. In patients with unilateral resting tremor the thalamus shows a significant grey matter increase contralateral to the tremor side, a phenomenon not evident in control subjects (Kassubek, et al., 2002).

VBM is advantageous over native space analysis in that it allows for identification of individual voxels that change within an area of interest. Each voxel that shows a change can be identified with provision also made for change to be shown if only large aggregations of adjacent voxels are also changed. In the thalamus, large clusters of voxels that have changed normally approximate a thalamic nucleus. This has been demonstrated in the PD-D group examined by Beyer & Janvin, et al., (2007) who reported grey matter reduction in a cluster of voxels in the pulvinar region in the absence of the wider thalamus. The mediodorsal nucleus of the thalamus has also been implicated in this way in PD patients with depression (Cardoso, et al., 2009), discussed in *Section 4.5.1.4*. In both studies VBM has allowed for identification of areas of change that would have been missed using native space analysis as, in both cases changes were only focal and the whole thalamus did not show any atrophy.

7.3.7 Diffusion and VBM

Four studies so far have also applied the voxel based method to diffusion tensor images. This combination of methodology enables detection of the subtle cellular changes that DTI is sensitive to, but in standardised space. The frontal and temporal degeneration typically identifiable in PD using traditional VBM methods (Beyer, Larsen, et al., 2007; Song, et al., 2011) is able to be detected much earlier on in the disease stage using diffusion VBM. Significantly decreased fibre directionality (a measure of axonal integrity) is evident in the frontal and temporal regions of non demented PD patients with short (6 years) disease duration (Kendi, et al., 2008). Axonal integrity is likley the first step of degeneration as no grey, or white matter loss was evident in any region of these patients. Diffusion changes within the frontal lobes were greatest in the supplementary motor area, prefrontal areas and the anterior cingulate. Although the PD patients were reported as having 'no cognitive impairment' this was only measured using the MMSE and, on average patients were scoring lower than the optimal 30 points (28.2) so could have had some slight cognitive impairment that went undetected, and this could be associated with the subtle changes detected. The idea that the fibre changes are influencing cognition is supported by the fact that, with exception to the motor area of the cortex, all other regions that showed change have previously been implicated in other studies of cognitive impairment. The anterior cingulate and prefrontal areas (Nagano-Saito, et al., 2005b) are commonly involved in executive function and memory deficits (Aarsland, Bronnick, Larsen, Tysnes, & Alves, 2009) in early and late stage Parkinson's disease. Lewy body density in the anterior cingulate has also been shown to be predictive of later cognitive deficits in Parkinson's disease (Kovari, et al., 2003).

Prior to the development of motor symptoms or cognitive impairment, early olfactory changes are considered to be some of the earliest symptoms of PD (Doty, Stern, Pfeiffer, Gollomp, & Hurtig, 1992). Combination DTI and VBM has been used to detect the neurocorrelates of olfactory deficits in early PD. The first study (Ibarretxe-Bilbao, et al., 2010) to identify disruption in the olfactory system using this method showed a significant decrease in fibre directionality (FA) and cellular integrity (MD) of the cerebeller and orbifrontal cortex of PD compared to control subjects. Disease duration of this patient group was on average, 3 years and motor dysfunction was at Hoehn and Yahr stage I and II so patients would not have been exhibiting a great degree of motor symptoms. Olfactory changes may therefore be evident prior to even the earliest clinical

presentation of motor dysfunction in some cases. Change in the rest of the olfactory system were later confirmed by Zhang, et al., (2011) who established that decreases in the fibre directionality within the cerebeller and orbifrontal cortex of PD patients corresponds to clinical measures of olfactory dysfunction. The degree to which these areas change can also be used as early biomarkers of PD and are able to correctly identify PD from control subjects in 94.1% of cases (Scherfler, et al., 2006).

7.4 Summary

Voxel based morphometry methodology allows for the automated examination of the whole brain without reliance on controlling for variance between users or the need for a prior hypothesis (Ashburner, et al., 2003). VBM has been applied in various PD samples, identifying changes in large areas of cortex and some subcortical regions. Implicated areas include frontal, temporal and occipital regions (Lee, et al., 2010) and, subcortically the regions within the temporal lobe (Ibarretxe-Bilbao, et al., 2009). Changes are more extreme and evident in larger areas in PD groups who also have dementia (Beyer, Janvin, et al., 2007), and degeneration co-varies with cognitive decline in longitudinal studies of PD cohorts (Burton, et al., 2005). Typically, grey matter degeneration is the most common finding in PD and in PD-D, but in some cases white matter degeneration has also been identified and is a reliable biomarker olfactory dysfunction (Scherfler, et al., 2006). Grey matter degeneration has also been found in the thalamus in some PD samples and is most extreme in those cohorts who have advanced cognitive decline (Burton, et al., 2004; Summerfield, et al., 2005). In contrast to what is able to be achieved in native space, VBM enables detection of sub regions of change within wider regions of interest. This allows for the identification of change that is not able to be measured from the gross volume analysis available in native space. Voxel based methodology can also be applied to diffusion images to detect the most subtle of cellular changes. Few studies have applied this method to PD cohorts, but in those that have, diffusion measures reveal significant cellular disruption in regions that later show significant white or grey matter degeneration (Karagulle Kendi, et al., 2008). Despite this methodology being able to detect early, subtle changes that covary with some of the earliest symptoms of PD this method has not yet been applied in the thalamus of PD patients in relation to cognitive dysfunction.

7.5 Hypothesis

- That results in standard space will reflect those identified in native subject space and the integrity of the thalamus will reflect cognitive dysfunction in Parkinson's disease

7.6 Method

7.6.1 Participant information

All participants (*Section 5.2*) were included in this analysis, the final sample consisted of images from 92 Parkinson's patients, of these 17 were classified as PD-D, 19 as PD-MCI and 56 as PD-N. All 25 control subjects were also included.

7.6.2 Imaging pre-processing

The structural (T1) and diffusion (MD and FA) images were used for the voxel based analysis. All VBM processing was completed using SPM 5 (Friston, 2005) in Matlab R2008a (Massachusetts, USA: The Math Works, INC) using the following steps.

7.6.3 Image analysis

7.6.3.1 Standardised elderly template

Due to the age of our participants a probabilistic template (Lemaître, et al., 2005) based on an elderly sample of 331 males and 331 females (age 65-72) was used for the segmentation procedure to reduce age bias and ensure more accurate segmentation and normalisation.

7.6.3.2 Segmentation

Unified segmentation (SPM 5) was used to segment images. The segmentation process classifies brain tissue into three tissue types: grey matter, white matter or cerebral spinal fluid (*Figure 7-1*) based on the prior probability of any voxel in a subject's image belonging to any of the tissue classes. This is able to be achieved irrespective of tissue intensity. The standard probability maps are generated by registering a large number of subjects together, assigning all voxels to the different tissue types and averaging the tissue classes over subjects.

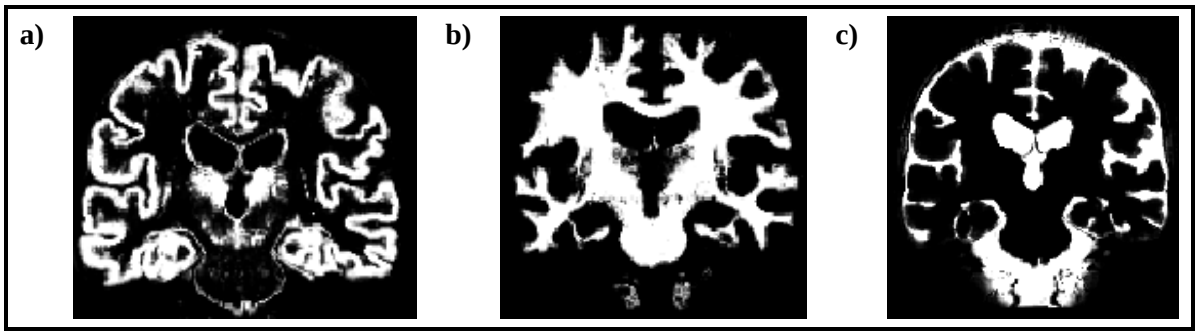


Figure 7-1: Segmented whole brain images
a) grey matter, **b)** white matter, **c)** cerebral spinal fluid segmentation.

7.6.3.3 Normalisation

The normalisation procedure involves assigning images stereotactically to standard space – in this instance subject images were normalised to the elderly template during the segmentation procedure. The primary function of this procedure is to enable inter-subject averaging and characterisation of anatomy as all individual subject regions are assigned to the same location in space as each other. Normalisation involves matching the study images to the template image using a series of algorithms. Normalisation begins with registration. The whole of the head is first matched to the template then only the cortex is matched together – using appropriate weighting of the voxels of the template. This procedure is automated and accounts for the confounding effects of skull and scalp differences, searching for the solution that maximises the probability of the registration being correct.

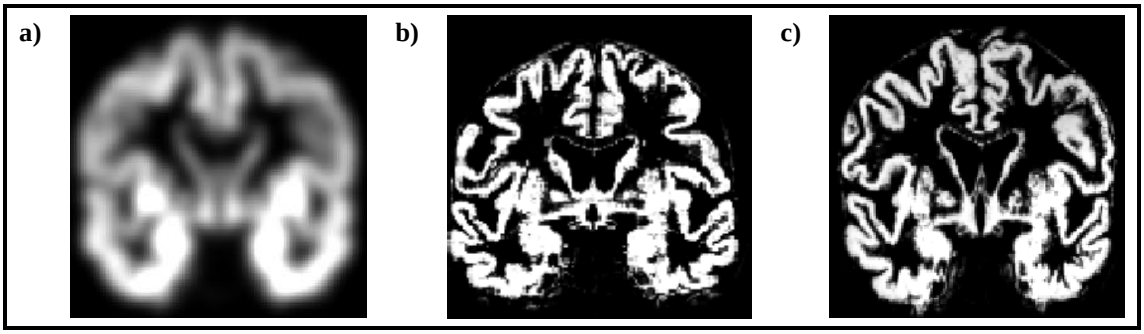


Figure 7-2: Normalised whole brain images
a) Grey matter , **b)** Normalised and modulated, **c)** Normalised, modulated and smoothed.

7.6.3.4 Modulation

The segmented and normalised images were modulated to compensate for the effect of normalisation, as when a series of images are warped to match a template, volumetric differences are introduced into the warped images. Modulation of the grey matter images preserves the volume of tissue within each voxel (Burton, et al., 2004). The procedure

involves adjustment of the signal proportional to the amount of warping by simply multiplying the relative volume of the normalised images before and after the warping procedure. The total amount of grey matter signal is now preserved in the resulting images irrespective of any volume changes that may have occurred in the previous steps.

7.6.3.5 Smoothing

The template and source images should have approximately the same smoothness in order for the data to be normally distributed and allow for statistical analysis to be applied (Ashburner & Friston, 2000). The elderly template has been smoothed by 8mm so the segmented, normalised and modulated images were smoothed using a 8mm FWHM isotropic Gaussian kernel to improve the signal to noise ratio.

7.6.4 Creation of the thalamus template

In order to restrict analysis to only the thalamus, a study specific template was created by averaging 20 previously defined thalamus images (n = 5 from each group).

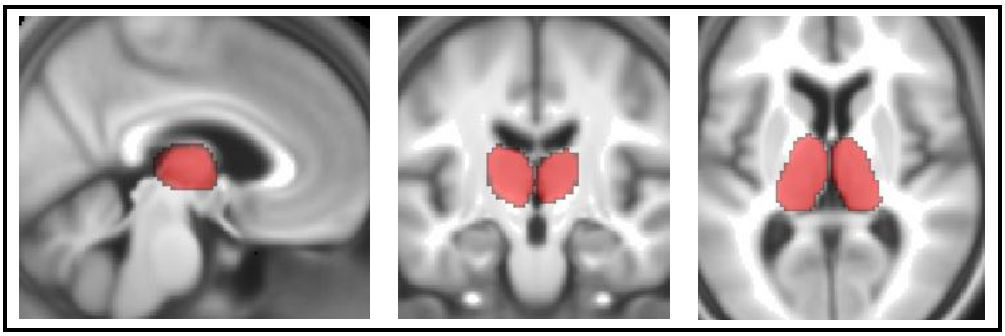


Figure 7-3: The thalamus template
A study specific thalamus mask was created and VBM analysis restricted to this area only. The template is seen here overlayed on the standardised elderly template.

7.6.5 Statistical Analysis

Statistical analysis for participant characteristics are described earlier (*Section 3.3.1*). Smoothed images from the pre-processing steps were analysed using SPM 5 (Friston, 2005). SPM is based on the general linear model and identifies regions of tissue with increased or decreased density or concentration that is significantly related to the effects being studied (Ashburner & Friston, 2000). The following voxel-based analysis (VBA) of grey and white matter, mean diffusivity and fractional anisotropy were performed using this analysis.

Differences between groups were assessed using between and within analysis of variance (ANOVA). Additional analysis of co-variance were also performed where age,

education and depression were included as co-variates in the first instance before excluding control subjects in order to focus on the changes within Parkinson's disease. Analyses restricted to Parkinson's disease patients co-varied for the disease specific variables disease duration and UPDRS III in addition to the original variables.

Multiple regression analyses examined the relationship between grey matter concentration and multiple measures of cognition using the general linear modelling feature of SPM5 which allows for all study images to be entered into the analysis along with the desired co-variates. The relationship between the intensity of each voxel and the cognitive measures were examined accounting for the following co-variates: age, education, depression, disease duration. The same model was applied to examine the relationship between cognition and white matter, mean diffusivity and fractional anisotropy voxel values. The cognitive domains examined were the same as those previously: attention/working memory/processing speed; executive function; visuospatial/visuoperception and learning and memory. Global Z Score, an aggregate measure of the above scores was also included. All results are presented at the voxel level. The co-ordinates for the anatomic location with maximal gray matter loss within each significant cluster are reported in MNI space.

To examine the influence of pre-processing steps, grey and white matter volumes of the thalamus in native space were extracted for each individual using Matlab 2008 (Massachusetts, USA: The Math Works, INC). ANOVA, ANCOVA and regression analysis were conducted in Statistica 2001 (Tulsa, USA: StatSoft, INC) following the same models with the same co-variates as above. In addition, Medcalc (Schoonjans, 1993) was used to create receiver operating characteristics of the grey and white matter measures to determine the discriminatory nature of the variables.

In all analyses only the thalamus was examined. Significance levels were set at $p < 0.05$ and corrected for multiple comparisons using the family wise error rate.

7.7 Results

7.7.1 Participant information

As all participants were included in this analysis demographic characteristics are identical to those reported in *Table 6-2.* In brief, the four groups were well matched in terms of education, depressive symptoms and the group distribution of sex and handedness. The PD-MCI and PD-D groups were older and had lower levels of premorbid IQ than the PD-N and control groups however. As expected, there was progressive worsening of clinical characteristics from PD-N to PD-MCI to PD-D. Cognitive characteristics are identical to those reported in *Table 6-3,* as before, the dementia group exhibited the lowest levels of cognitive function, the PD-N group a level within the range of that of healthy control subjects and the PD-MCI group a level that was generally intermediatery between the PD-N and PD-D groups.

7.7.2 Cross sectional changes in thalamic concentration

The grey matter and white matter concentration levels of the thalamus were compared between the two groups. To examine the similarity between the thalamus in native space and standardised space, mean diffusivity and fractional ainsotropy of the thalamus was also compared between groups. Grey and white matter values were extracted from the standardised images and examined using general linear modelling in Statistica. The mean diffusivity and fractional ainsotropy values were examined using voxel based analysis in Matlab.

7.7.2.1 Grey and white matter concentration

Mean grey and white matter concentration levels are shown in *Table 7-1* with mean values after inclusion of demographic covariates presented in *Figure 7-4.* With the exception of total grey matter volume in the dementia group which was slightly positively skewed (SW-W = 0.89, $p = 0.05$) all variables met the assumption of normality and the Levene's test for homogeneity of variance. There was no group or hemisphere effect for the level of grey matter concentration. Adding age, education and level of depressive symptoms did not change results [$F(3,110) = 0.29$, $p = 0.84$] nor did excluding control subjects and adding the clinical covariates disease duration and UPDRS [$F(2,84) = 0.10$, $p = 0.90$].

Table 7-1: Mean (SD) group differences in thalamic grey and white matter integrity

	Control (n = 25)	Unimpaired (n = 56)	MCI (n = 19)	Dementia (n = 17)	Group Effect		Hemisphere Effect	
					F	p	F	p
Grey Matter	8272.97 (1363.15)	8653.94 (1185.21)	8326.76 (836.04)	8469.62 (1449.63)	0.72	0.54	0.68	0.41
Left	4158.26 (675.18)	4331.15 (618.86)	4133.68 (424.22)	4287.27 (748.48)				
Right	4114.80 (697.66)	4322.85 (616.95)	4193.07 (443.04)	4182.34 (720.42)				
White Matter	4994.83 (970.13)	5251.78 (1276.95)	4802.56 (1343.54)	4865.08 (1070.18)	0.94	0.42	52.77	<0.001
Left	2581.52 (474.73)	2770.01 (702.12)	2521.36 (716.77)	2552.67 (548.13)				
Right	2413.32 (545.20)	2481.78 (621.30)	2281.23 (645.41)	2312.39 (539.37)				

Grey and white matter concentration is reported mean (SD) in mm^3. There was no group effect and no hemisphere x group interactions for either measure.

There was also no group effect for white matter concentration in the thalamus, although there was a main effect of hemisphere in the left > right direction. There was no group x hemisphere interaction; the hemisphere effect was consistent for all groups. Adding covariates did not result in a group effect [$F(3,110) = 0.49$, $p = 0.69$] and reduced the hemisphere effect so that it was no longer significant [$F(1,110) = 0.01$, $p = 0.92$]. Results did not change with the addition of clinical covariates [$F(2,84) = 0.48$, $p = 0.62$].

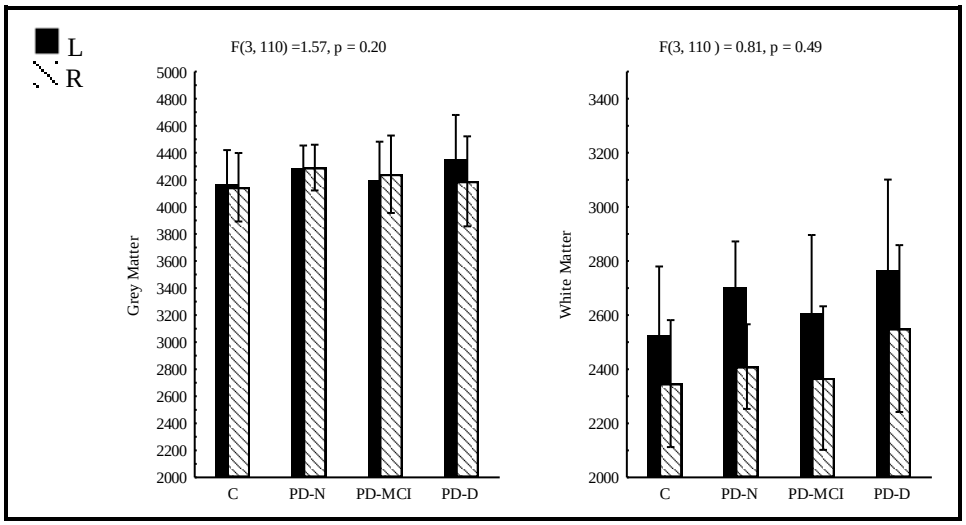


Figure 7-4: Grey and white matter concentration after controlling for covariates
There was no group difference in mean grey (**a**) and white (**b**) matter concentration values. Lines represent standard error bars.

7.7.2.1 Diffusion integrity changes

Mean diffusivity measures have previously been shown to be sensitive to cognitive group when the thalamus was examined in native space (*Section 6.7.4*). To investigate whether this result held when the thalamus was examined in standard space several pairwise comparisons were made using VBM analysis for all groups on both FA and MD measures *(Figure 7-5*). Results were consistent with the native space results. Mean diffusivity was again sensitive to group at the voxel level, the PD-D group exhibited increased diffusivity in several thalamic areas relative to all other groups.

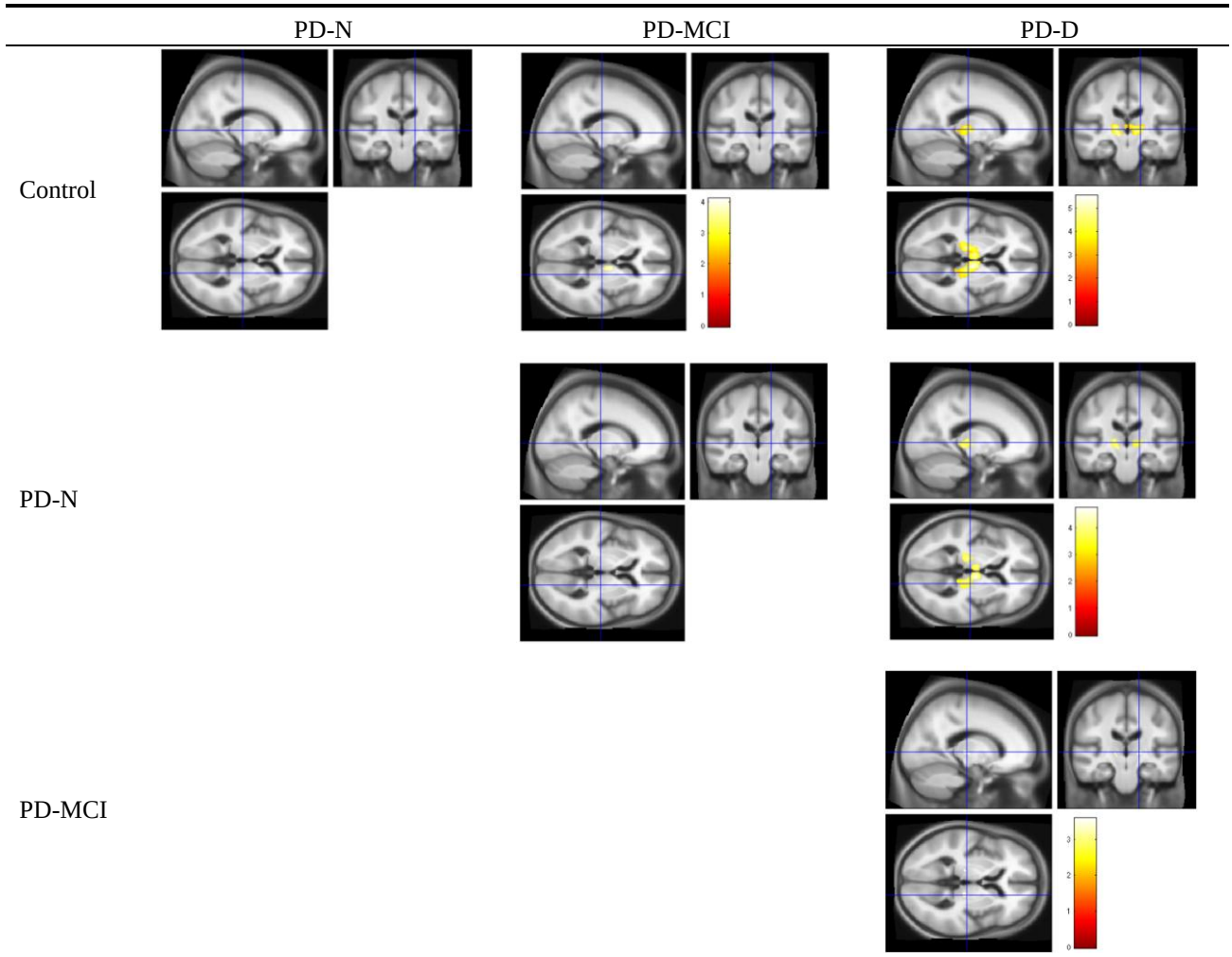


Figure 7-5: Pariwise comparison of control and PD-D group.
Areas of significantly increased mean diffusivity ($p < 0.01$) are overlayed on the mean brain T1 template in saggital, coronal and axial view. The blue crosshair is positioned onto ventral posterior lateral thalamus (MNI co-ordinates 16, -20, 2).

The size and coordinates of each area are reported in (*Table 7-2).* The ventral regions of the thalamus were particularly susceptible; the largest areas of thalamic change were between the PD-D and the control and PD-N groups. A smaller thalamic area also showed increased mean diffusivity in the PD-D group relative to the PD-MCI group. Fractional ainsotropy was not reduced in any group.

Table 7-2: Areas of significant difference in MD within the thalamus

MNI coordinates			Area	T value	FWE Corrected (p<0.05)
x	y	z			
C vs PD-D					
8	-12	0	Ventral Anterior	5.18	<0.001
-12	-22	-6	Ventral Lateral	5.16	<0.001
-16	-22	4	Centromedian/Parafascicular	5.06	0.001
-8	-10	-2	Centromedian/Parafascicular	4.64	0.003
16	-20	2	Ventral posterior lateral	4.36	0.007
20	-24	6	Ventral posterior lateral	4.10	0.017
20	-26	-2	Ventral posterior lateral	3.78	0.045
PD-N vs PD-D					
8	-12	0	Ventral Anterior	4.46	0.005
5	-12	4	Centromedian/Parafascicular	4.41	0.005
-5	-10	-6	Ventral Anterior	4.29	0.009
-2	-12	5	Mediodorsal	3.91	0.030
18	-24	5	Ventral Lateral	3.85	0.037
14	-24	4	Centromedian/Parafascicular	3.82	0.040
20	-28	-2	Ventral posterior lateral	3.68	0.059
PD-MCI vs PD-D					
16	-20	2	Ventral Lateral	3.79	0.042
-14	-22	-4	Centromedian/Parafascicular	3.75	0.048

The regions of the thalamus that show significantly increased mean diffusivity. **FWE**: family wise error

7.7.3 The association between the integrity of the standardised thalamus and cognition

The relationship between thalamic measures and cognition was examined in Matlab with multiple regression analyses. The relationship between each thalamic measure and cognitive domain was examined separately for control participants and PD patients. In the models for the control subjects age, education and depression were included as covariates. There was no relationship between any measures of the thalamus and cognition within the control group (*Table 7-3*). The additional covariate disease duration was added when multiple regressions were restricted to patients only. Similar to the regression results for the thalamus in native space (*Section 6.7.5*) mean diffusivity measures of the thalamus were more sensitive to cognition than the other diffusion or integrity measures. Mean diffusivity measures in large areas of the thalamus had a strong correlation with overall global cognition and all individual cognitive domains except the learning and memory domain (*Table 7-3*). White matter concentration and fractional ainsotropy did not have an association with any cognitive subset.

Table 7-3: Size (mm^3) of thalamic clusters which have a significant relationship with cognition

	Global Z Score		Attention/WM/ Processing Speed		Executive Function		Visuoperception/ Visuospatial		Learning and Memory	
	Size	T (*p* value)	Size	T (*p* value)	Size	T (*p* value)	Size	T (*p* value)	Size	T (*p* value)
Control Subjects										
Grey Matter	0		0		0		0		0	
White Matter	0		0		0		0		0	
Mean Diffusivity	0		0		0		0		0	
Fractional Anisotropy	0		0		0		0		0	
PD Patients										
Grey Matter	0		0		0		0		0	
White Matter	0		0		0		0		0	
Mean Diffusivity	256	4.23, (0.02)	112	4.03 (0.03)	552	4.32 (0.03)	96	3.97 (0.03)	0	
MNI co-ordinates	6	-14 2	6	-14 0	6	-14 0	8	-22 2		
Area	CM/Pf		CM/Pf		CM/Pf		CM/Pf			
Fractional Anisotropy	0		0		0		0		0	

The relationship between thalamic measures and cognition. Size of the area of the thalamus (mm^3) which has an association with each measure after accounting for the co-variates: age, education and depression in the case of control subjects with disease duration an additional covariate added for the PD patients models. P values are reported after family wise error correction. **MNI**: Montreal Neurological Institute; **CM/Pf:** Centromedian/Parafascicular region.

7.8 Discussion

7.8.1 Summary of the results

We expected the standard space examination to reflect the results of the native space study in that thalamic degeneration would reflect cognitive dysfunction in Parkinson's disease. This hypothesis was only partly supported. When mapped to standard space, the thalamus does not show any reduction in integrity across the cognitive spectrum of Parkinson's disease despite the results of the native space analysis indicating a volume reduction in PD-D. Diffusion tensor image results, on the other hand are more sensitive to cognitive impairment and reflect native space results, there is a significant disruption in cellular integrity in the PD-D group compared to healthy control subjects and the PD patients without cognitive impairment. VBA allows for the identification of regions of change within the thalamus instead of only detecting thalamic change if it induces atrophy of the whole structure. Along with the motor regions of the thalamus, the ventral posterior, centromedian and mediodorsal regions all showed significant cellular disruption. The non-specific centromedian/parafascicular region had the greatest degree of change, and after accounting for covariates was heavily implicated in all aspects of cognition except learning and memory. The centromedian/parafascicular region was the only region that was implicated in cognition however, no other regions had an association with any cognitive domains.

7.8.2 Standard space results compared to native space results

Comparisons between the methodology used in this chapter and the one previous yielded different results, the level of change detected in the thalamus in standard space is not as great as when the thalamus is examined in native subject space. The thalamus shows no change in white or grey matter integrity between any groups in this study. The lack of thalamic change is consistent with previous VBM results reported from our own group (Melzer, et al., 2011b) but in contrast to previous results which do show a reduction in PD-D relative to control subjects in the bilateral (Burton, et al., 2004) and left (Summerfield, et al., 2005) thalamus and a reduction in PD dementia relative to those PD without dementia in the bilateral (Nagano-Saito, et al., 2005b) and right pulvinar region of the thalamus (Beyer, Janvin, et al., 2007). Our results are mostly consistent with Peran, et al., (2010) who is the only other study to apply both standard VBM and native space analysis to the

thalamus of the same PD sample. In the Peran, et al., cohort, the authors determined that the regions were accurately assessed in all instances and the subcortical structures created on the VBM map did not show any noteworthy variations between subjects compared to manually defined regions. Results were mostly the same as seen in our cohort; mean diffusivity of the thalamus was significantly increased in PD relative to controls, when examined in both standard and native space. Fractional anisotropy results were in contrast to ours however, FA was reduced in the left thalamus in both VB and native space analyses but was only showed reduction in the right thalamus in native space.

The reason for the discrepancy between our results and those of previous studies is unlikely to be due to methodological differences as the protocol used here is the optimised VBM protocol created by (Good, et al., 2001) which was also applied in all the above studies except the one conducted by (Nagano-Saito, et al., 2005b).

The results here reflect examination of the thalamus only, rather than the whole brain approach which was used in all previous studies, which could account for discrepencies. After the normal image analysis processing we created a template of the thalamus using a subset of patients from each cognitive group and restricted statistical analysis to this area only. A similar method was used in Summerfield, et al., (2005) where volumetric analysis was restricted to the wider temporal lobe area (caudate, lentiform nuclei, cingulate gyrus, anterior cingulate, thalami, insula, extranuclear region, amygdala, hippocampi, and the parahippocampus gyri). When comparing the results of these two studies the whole brain approach appears to be best for detecting gross regions of atrophy such as in the cortex of the temporal or frontal lobes. It is only when these areas are excluded from the analysis that further changes in smaller regions are evident.

When the wider cortex of the temporal lobe was excluded in the (Summerfield, et al., 2005) analysis for example, further subcortical changes that had not been previously identified were evident. Larger areas of the hippocampus and the putamen, accumbens, hypothalamus and thalamus also showed significant grey matter reduction.

In relation to our own whole brain VBM analyses, there is no thalamic change (Melzer, et al., 2011b). This is despite the sample size of patients being more than adequate and patients of a similar level of impairment as the previous studies. The patients included in the Melzer, et al., (2011b) study are also very similar to the ones that have been examined in this thesis, indicating the similarity between our results and the dissimilarity with others may, in fact be due to the patient sample. As, in both studies from our group the patients were of lower cognitive state, had a higher Hoehn and Yahr score and were

older than the others it is unlikely they were not showing significant levels of impairment however.

VBM of the whole brain has been compared directly with a region of interest approach, using both an automated and manual method in standardised space (Kennedy, et al., 2009). Manual tracing methods are considered to be the gold standard in neuroanatomy analyses, and compared to manual tracing, whole brain VBM produced stronger differences between subject groups and a different spatial distribution of changes compared to manual measures. This suggests that the whole brain VBM approach has a high propensity for Type II error. When VBM was restricted to previously defined anatomical regions however, the gross differences between VBM and manual methodology disappeared suggesting that it is not the VBM methodology itself that is prone to error, rather, spurious results are likely when a large area is examined for change.

The Kennedy, et al., (2009) study was conducted on a large cross-sectional sample of healthy non demented adults to examine the relationship between age and cortical grey matter reductions. The optimised VBM protocol of Good, et al., (2001) was applied for the whole brain VBM analysis. Study specific masks for subcortical structures were manually drawn on the T1 coronal image of one patient and then registered to standard space, a method similar to the one applied in this thesis. Unfortunately the thalamus was not included in the masking procedure and cortical regions of interest were restricted to the anterior cingulate gyrus, dorsolateral prefrontal cortex, temporal and parietal regions. Subcortically, only the hippocampus and parahippocampal gyrus were included.

VBM whole brain results showed a widespread and less differential pattern of age differences than the manual volumetric method. Furthermore, a direct comparison between manual tracing and VBM derived masks of subcortical structures revealed that, for some regions the whole brain VBM actually produced isolated outliers that enhanced the association with age. This elevated sensitivity of whole brain VBM suggests that the positive findings of regions of change for VBM results should not be taken at face value. The reason for the occurrence of spurious results is thought to be due to the spatial smoothing of an image. When an image is spatially smoothed the resolution of that image is consequently reduced, serving to average smaller structures in such a way that small differences are amplified (Allen, Bruss, Brown, & Damasio, 2005).

When just the hippocampus and parahippocampal gyrus were compared using the previously defined masks in the above study however, the discrepancy between whole brain and manual methods disappeared. VBM, therefore does seem to provide realistic

estimates of differences in regional grey matter, but only when applied to masked regions rather than whole brain regions. The authors recommend that whole brain VBM analysis be used as a hypotheses-generating method. Any areas of difference that are identified should then be explored further using masking or manual tracing methods. In relation to our results, the contrast between our report of no grey matter reduction in the thalamus and the grey matter reduction that others have identified could be due to the fact that the whole brain VBM approach has lead to misleading results.

One study does show a similarity between whole brain and masked VBM approaches, with the sensitivity of the masked VBM approach increased in the thalamus. In patients with chronic pain the thalamus shows a similar level of grey matter atrophy in both a whole brain and masking VBM approach (Gwilym, Filippini, Douaud, Carr, & Tracey). Initially, the authors conducted a whole brain VBM analysis to examine neurocorrelates of pain in patients with osteoarthritis of the hip. The thalamus was one of the regions implicated in this initial analysis but the significance of the comparison between pain patients and healthy controls disappeared after provision for multiple comparisons were made. The thalamus was then isolated using a masking approach. Results were similar between the two methods, when masked from the whole image the thalamus showed a significant reduction in the pain patients and the result remained significant as multiple comparisons did not need to be taken into consideration. In both cases the change was isolated to the bilateral medial thalamus which has been implicated in pain processing in only a few cases before (Apkarian, et al., 2004).

Our discrepancy between manual and VBM masked results is therefore most likely due to the differences in the underlying tissue that is measured using the two methods. VBM is restricted to measuring one tissue type at a time (Good, et al., 2001). Voxel based measures in standard space only take into account white and grey matter where white matter tissue primarily consists of the axons of a cell body and grey matter is the cell bodies themselves, along with dendrites and other extracellular and intracellular structures that form the total cell structure (Kanai & Rees, 2011). Volumetric measures of atrophy on the other hand take into account all cellular structures and all tissue types. The fact that we found no grey matter, or white matter reductions in the thalamus in this sample could suggest that the overall atrophy levels found in the manual tracing sample are reflective of the cumulative degeneration of total neurons – structures which are reflected in both grey and white matter. Although degree of neuronal degeneration does normally correspond with the degree of volume loss observed in the thalamus of PD patients, this is only true for

some of the thalamic nuclei (Halliday, 2009; Henderson, et al., 2000a). The lack of grey matter reduction identified in our sample therefore could indicate that neuronal loss alone is not occurring to a degree that is great enough to be detected by VBM. The volume loss identified in native space study could be reflective of the degeneration of extracellular structures alongside neuronal loss and could account for the differences in results between the two methodologies. This idea is supported by the fact that diffusion tensor analysis was more sensitive to cognitive dysfunction in PD than grey matter analysis in this sample, in line with results seen in our native space study (*Section 6.7.4*). Although there were no grey or white matter changes between any of the PD groups, mean diffusivity images showed significant differences between PD-D and all other groups.

One VBM study has also applied region of interest guided VBM of white matter maps of the thalamus in Parkinson's disease with the objective of comparing patients with and without co-morbid depression (Li, et al., 2010). Decreased FA was evident bilaterally in the patients with depression and PD compared to those PD patients without depression. The region most implicated was the mediodorsal region of the thalamus. This result is in contrast to our VBM analysis where no differences were found in the white matter of the thalamus – most likely due to the fact the patients in the (Li, et al., 2010) sample were of normal cognition and the patients in our sample were not showing any depressive symptoms.

No other studies have examined the thalamus in standard space in PD. Our results that diffusion measures are disrupted in the absence of any integrity measures are somewhat supported by previous research in other cortical regions of PD however. Fiber integrity in temporal and frontal regions is disrupted in early PD (Karagulle Kendi, et al., 2008) and regions of the olfactory system also show significant diffusion changes in PD in the absence of any integrity changes (Ibarretxe-Bilbao, et al., 2010; Scherfler, et al., 2006; Zhang, et al., 2011). Analysis of diffusion tensor images allows for the earlier detection of change than grey or white matter integrity in standard space.

In line with the discrepancy between volume and integrity measures of the thalamus, DTI results in standard space are not as sensitive to cognition as DTI results in native space. Although fractional anisotropy measures showed no change between groups in either case, mean diffusivity showed progressive increases across the cognitive spectrum of PD in the native, but not standard space analysis. Diffusivity in the PD-MCI group was increased relative to PD-N in native space for example, and a further increase was shown in the PD-D group relative to PD-MCI. In contrast, the standard space results only

revealed a change in diffusivity once cognitive impairment was extreme, showing an increase in PD-D relative to PD-MCI but not in PD-MCI relative to PD-N. The discrepancy between the two studies is likely an artifact of the VBM methodology as several other studies also report less sensitivity when interpreting small structures using VBM (Brookstein, 2001; Davatzikos, 2004; Kennedy, et al., 2009).

7.8.3 The influence of the thalamus on cognition from standard space examination

Grey matter changes did not support the hypothesis, there no areas of grey matter change in the thalamus and no association between grey matter volume and any aspect of cognition in our sample. It is difficult to draw conclusions about this in PD as previous studies have either not conduced analysis (Beyer, Larsen, et al., 2007; Burton, et al., 2004; Summerfield, et al., 2005) or report no relationship (Dalaker, et al., 2010; Nagano-Saito, et al., 2005b) between thalamic grey matter and cognition. The above studies have implicated the thalamus in PD-D relative to control subjects and in PD-D relative to PD-N however, so by virtue of the fact that the thalamus shows a reduction in the group with cognitive dysfunction our results could be considered to be in contrast to this.

As the bilateral thalamus has also been implicated in mild cognitive impairment of the Alzheimer's type (Pennanen, et al., 2005; Teipel, et al., 2010), this suggests that there could be some association with the thalamus in PD patients with cognitive impairment. In the Pennanen, (2005) sample the thalamus was significantly reduced relative to control subjects. No statistical analysis was conducted to determine the influence this had on cognition but it could be assumed that the thalamus was at least partially influential on the development of AD symptoms. In the Teipel, (2010) study the influence on grey matter atrophy in the thalamus was additionally examined in patients who converted to AD at follow up and MCI patients who had not converted to AD at follow up. The thalamus was one of the only regions of subcortical atrophy identified in the MCI subjects who later converted to AD. The thalamus was also implicated in another AD study (Chetelat, et al., 2005) and showed a significant level of grey matter reduction in the patients that converted from MCI to AD.

The contrast between findings of thalamic involvement in PD and AD could be due to confounds of the motor dysfunction of PD as one study (Kassubek, et al., 2002) reports an increase in the grey matter of the thalamus in PD as a result of motor dysfunction. The grey matter increase was reported in the hemisphere contralateral to the side PD movement

symptoms originated in, suggesting this was due to compensatory mechanisms. If grey matter increases reflect movement dysfunction it can only be assumed that, when analysed with VBM this serves to 'cancel out' any grey matter decreases that may be occurring as a function of cognitive dysfunction.

In terms of the mean diffusivity changes in the thalamus in PD, the second hypothesis was supported. We have shown for the first time that areas of change were restricted to only some regions of the thalamus and were mainly isolated to the motor ventral anterior and lateral nuclei and to the association mediodorsal nucleus and the non-specific centromedian/parfascicular area. Diffusivity was significantly increased in these areas of PD-D relative to all other groups.

Our VBM study did not allow for analysis of the relationship between each thalamic region and cognition and, overall, results only partially suggest a domain-specific influence on cognition. In total, mean diffusivity measures of the thalamus show an association with executive function, attention, visuospatial/visuoperception function and the aggregate global Z score. The only cognitive domain not implicated was learning and memory. The relationship between diffusion measures of the thalamus and executive function is somewhat in accordance with the results of (Rose, McMahon, et al., 2006) who report that diffusion measures of the right thalamus are significantly correlated with measures of executive function in participants with MCI of the Alzheimer's type. In contrast to our mean diffusivity results however, the diffusion measure used in the Rose, McMahon, et al., (2006) sample was fractional anisotropy rather than mean diffusivity and our sample shows no change in fractional anisotropy measures.

In regards to the regions within the thalamus, the mediodorsal nucleus has previously been extensively implicated in performance on attention, memory and executive function tasks in Schizophrenia (Kemether, et al., 2003) and it is therefore somewhat surprising that no relationship with memory is evident in our sample. It is also surprising that the ventral anterior and ventral lateral nuclei are both implicated in cognition here, as traditionally they are considered to be pure motor influences (Jones, 2007a) and analyses was conducted here independently of disease duration effects which may have otherwise accounted for this result.

The centromedian/parafascicular association with cognition was the only expected result. This region has traditionally been considered 'non-specific' (Jones, 2007a) but has also been implicated in some of the frontal symptoms of Schizophrenia, including executive function deficits (Kemether, et al., 2003).

The fact that learning and memory components of cognition are not implicated here could be due to the fact that this is commonly considered to be an anterior dominant (Aggleton & Brown, 1999) function and the anterior region of the thalamus showed no changes in diffusivity.

Other regions of the cortex that are known to be involved in cognition have been implicated using VBM however, suggesting that the lack of thalamic findings here are most likely due to the difficulty that arises when examining small structures in VBM (Kennedy, et al., 2009) – not due to a lack of cortical change in our PD sample.

7.8.4 Limitations of the study methodology

Limitations inherent to whole brain VBM studies include confounds that arise from warping structures and cortices to the same space; a high risk of reporting false positive findings and difficulty in interpreting the results of small structures (Bookstein, 2001; Crum, Griffin, Hill, & Hawkes, 2003). Registration to standard space has also been shown to work least well in groups where there are large differences in structures (Kennedy, et al., 2009) – particularly around the ventricles. As Parkinson's disease patients typically have enlarged ventricles (Dalaker, et al., 2011) it was especially important to take this into consideration when conducting voxel based methods on this sample. We hoped to overcome the majority of difficulties that typically arise during the whole brain VBM approach by masking out the thalamus from the standard space image and using a region of interest approach.

Here, the region of interest approach shows no grey matter reduction of the thalamus in Parkinson's disease; although this has been identified in previous research. There are two possibilities for this. Firstly, that previous studies are reporting false positives or secondly, that the region of interest approach is not sensitive to thalamic destruction in Parkinson's disease. Due to the fact our group has previously used the optimised protocol (Good, et al., 2001) in a similar PD cohort and also found no thalamic changes (Melzer, et al., 2011b) the second hypothesis is unlikely. Some previous studies have attempted a direct comparison between a region of interest approach and whole brain analysis. One study found an increase in false positives when the whole brain approach was applied (Kennedy, et al., 2009) and the other, no difference between the two methods (Gwilym, et al.). We conclude, therefore that the masked region of interest approach is comparable, if not better to the whole brain VBM approach. As mean diffusivity results are similar to those we report in native space (*Section 6.7.4*) this provides further evidence

that the region of interest approach is more sensitive to 'real' thalamic degeneration and less prone to Type II error than the whole brain approach. That is, grey matter density changes may not have been identified here as thalamic disruption may not have been to a degree great enough across the board of the thalamus to be reflected in an examination of the whole thalamus, a hypothesis that will be explored further in the subsequent chapter.

7.8.5 Summary

The thalamus was examined in standard space using a region of interest approach. No grey matter reduction was found in the thalamus, a result that is in contrast to previous reports of thalamic reduction that has been identified using a whole brain approach (Beyer & Aarsland, 2008; Summerfield, et al., 2005). Our own group, however has reported no thalamic changes using a whole brain approach in a similar sample (Melzer, et al., 2011b). We suggest that the discrepancy between the findings of our own group and others is due to methodological, and patient cohort differences. Although the criteria of Good, et al., (2001) was applied across the board of studies we have segmented the thalamus from the whole brain and only applied the VBM methodology to this masked image rather than the whole image. This method has been applied previously in other samples and found to reduce false positive findings (Kennedy, et al., 2009) or show no difference between this and the whole brain approach (Gwilym, et al., 2010).

We conclude, therefore that the lack of grey matter reduction seen here in the thalamus, although in contrast to our own native space results (*Figure 6-6*) is a more likely finding than that that has been reported in pervious studies. As grey matter integrity reflects the neuronal body density and does not take into account the axonal white matter or other extracellular structures, we propose that neuronal density is not the only factor to take into consideration when examining native atrophy results. The atrophy that is evident in native space is likely to be reflective of all other cellular components as well as neuron bodies.

Chapter 8. Study Three: THE THALAMIC NUCLEI IN NATIVE SPACE

8.1 Objectives

The individual nuclei that comprise the thalamus are addressed in this chapter. The aim of this study is to examine the gross volumetric as well as the subtle cellular changes in clusters of the thalamus that are assumed to represent the main thalamic nuclei. Thalamic nuclei changes that have already been identified in PD or in patients with other types of cognitive decline are briefly reviewed here from a variety of research methods including post mortem neuro-histology and *in vivo* magnetic resonance imaging (MRI). The breakdown in cortico-thalamic connectivity will be addressed in the subsequent chapter (*Chapter 9*).

8.2 Nuclei involvement in PD, dementia and cognitive dysfunction

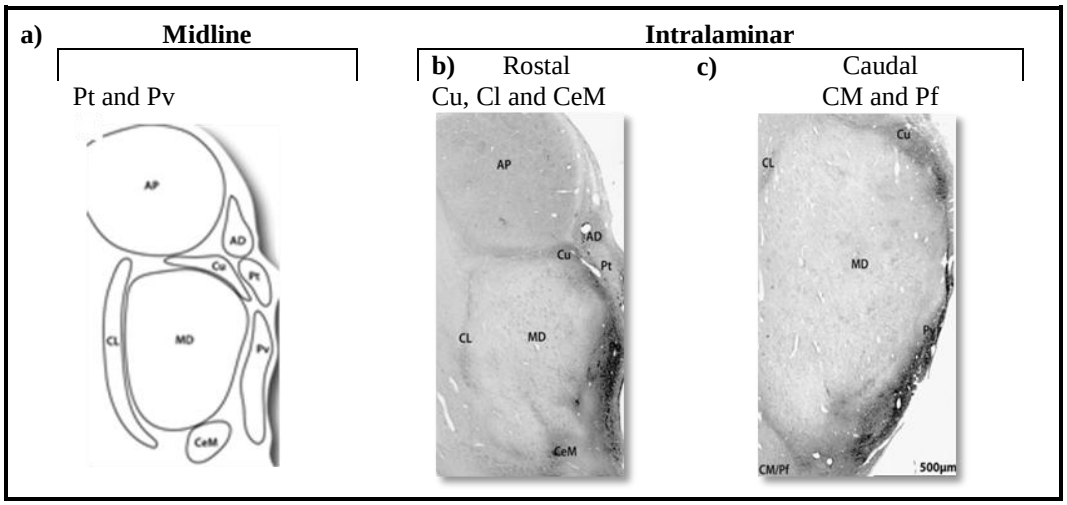
The cognitive symptoms of Parkinson's disease are heterogeneous, pointing to the involvement of several cortical areas. The structure – function relationship does not operate in isolation however and it is the interaction between cortical areas that is most important in Parkinson's disease. Braak (2003) suggests that the progression of Lewy pathology from preclinical, to symptomatic, to advanced disease spreads along axonal pathways that interconnect vulnerable brain regions. The multifaceted symptoms of PD likely arise from this cortical wide disruption in connectivity and communication, affecting normal function and behaviour to a greater degree than the sum of degeneration in isolated areas could. As the thalamic nuclei each show reciprocal connectivity with key areas of the cortex these subcortical structures are regions that are likely to reflect, or give rise to the degeneration that may be occurring in the wider cortex.

8.2.1 Parkinson's disease

The midline and intralaminar nuclei have been frequently examined in PD due to their connectivity with the striatum and areas of the motor cortex (Jones, 2007a), areas heavily involved in PD motor dysfunction (Brooks & Halliday, 2009). Degeneration in these regions is easily identified using both neuro-histology and *in vivo* MR methodology as this region of the thalamus can be visually identified from structural MR images with some success (Kemether, et al., 2003). There are several subdivisions within both the midline

and intralaminar nuclei which have been individually implicated in distinct subtypes of motor symptoms in PD. The midline nucleus consists of the patatenial nucleus (Pt) and the paraventricular (Pv) nucleus and lies dorsal to the larger intralaminar complex. The intralaminar complex is an aggregation of several smaller nuclei and lies in the ventral region of the thalamus and is easily distinguished from the surrounding thalamus due to the border of the internal medullary lamina. The intralaminar complex can be further distinguished into caudal and rostal components. Larger in size, the caudal region includes the CM/ while the rostal region is comprised of the smaller central dorsal, central lateral (Cl) and central medial (CeM) nuclei (*Figure 8-1*).

Figure 8-1: Location of the components of the midline and intralaminar nuclei



Coronal sections of the thalamus demonstrate the architecture of the midline (**a**) and intralaminar nuclei at the anterior (**b**) and posterior (**c**) ends of the thalamus. Image modified from (Brooks & Halliday 2009). **AP:** anterior principal; **AD:** anterior dorsal; **Cu:** cucullar; **Pt:** Paratenial; **CL:** central lateral; **MD:** mediodorsal; **Pv:** paraventricular; **CeM:** central medial; **CM/Pf:** centromedian/parafascicular.

To date, only one MR study (McKeown, et al., 2008) has identified degeneration in the intralaminar region of PD patients compared to control subjects. Although the volume of the whole thalamus was examined the only area of significant change was in the CM/Pf. The lack of total thalamic volume loss is consistent with other studies who report no overall thalamic volume reduction in PD patients (Peran, et al., 2010; Rizzo, et al., 2008), suggesting degeneration may only occur in select areas that are undetectable when measuring gross thalamic atrophy in PD patients without dementia. Although cell loss was not specifically investigated in the McKeown, et al., sample thalamic degeneration was hypothesised to be due to the loss of neuronal and glial cells.

Lewy pathology is in fact the likely cause of cell loss as cellular loss has been confirmed to occur as a function of the accumulation of the alpha synuclein (α-SN) protein

which is the primary component of Lewy body (LB) and Lewy neurites (LN) (Volpicelli-Daley, et al., 2011). These pathological hallmarks of Parkinson's disease (Baba, et al., 1998) differentially target specific thalamic nuclei and could result in cell loss in some regions only.

Several neuro-histological studies have identified heavy Lewy burden in some nuclei whilst others remain relatively spared. Lewy pathology heavily infiltrates the CM, and to a lesser degree the Pf nuclei in PD (Rub, Del Tredici, Schultz, et al., 2002) for example, occurring in the early stages of the disease and increasing significantly in those groups with cognitive impairment (Braak, Rub, et al., 2006).

Pathology is significantly related to the level of neuronal loss in PD. In an aggregation of PD patients (Halliday, 2009) from several different autopsy studies (n = 37) who had a long (13 years) disease duration but no dementia, neuronal and volume loss was associated with pathology levels in some thalamic regions. Components of the limbic loop (CL, CeM, Pt and Pv nuclei), an integral system for learning, memory and attention process, for example were heavy targets for PD-related pathology while the motor nuclei belonging to the striatal loop (CM, VA) were only lightly involved. Nuclei in the cerebellar (VL) loop – which connects the premotor and primary motor fields (Braak, et al., 2000) and the medial region of the sensory (VP) nuclei were only infiltrated by a few LB's or LN's. Not surprisingly, those regions that were heavily targeted by pathology also showed the greatest degree of neuronal loss. Neuronal reduction was evident in the Pv and Pt nucleus, and was so severe in the Pt nucleus that gross atrophy was also visible (Henderson, et al., 2000b). The posterior (VLp) and anterior (VLa) subdivisions of the VL nucleus and the VA nucleus, on the other hand did not exhibit any reduction in neurons, despite being critical components of the motor loop (Halliday, 2009). The VL nucleus has shown neuronal loss at trend level in another PD sample (Xuereb, et al., 1991) however.

The mediodorsal and anterior nuclei that primarily connect to the prefrontal and limbic cortices and the anterior cingulate cortex, respectively, were also included in the above works but did not show either neuron or volume loss (Halliday, 2009), although the AP nuclei was also implicated in the earlier work (Xuereb, et al., 1991) and reported to have an 80% reduction in neurons.

There is a strong relationship between α-synuclein deposits, neurodegeneration and the appearance of clinical (motor, autonomic and neuropsychiatric) symptoms in PD (Hawkes 2010). Total LB density is also significantly associated with the rate of annual decline in MMSE score (Aarsland, Perry, Brown, Larsen, & Ballard, 2005) and LB density

in frontal, temporal and associated subcortical (amygdala, hippocampus) regions correlates with a global deterioration scale of cognitive impairment, independently of Alzheimer's neuropathology (plaques and tangles) (Mattila, et al., 2000).

DTI images can be thought to be representative of underlying cellular integrity and density (Kinoshita, et al., 2008) and have confirmed that cell loss does not occur throughout the thalamus uniformly. In the only DTI study to date that has examined the regions of the thalamus in PD patients, differential loss was found in PD patients with co-morbid depression compared to those without (Li, et al., 2010). The mediodorsal nucleus (MDn) had significantly higher mean diffusivity in patients with co-morbid depression with no corresponding changes in any other regions of the thalamus.

8.2.2 Dementia

Several thalamic nuclei are implicated in Parkinson's disease dementia. The post-mortem work by Brooks & Halliday (2009) in Lewy body diseases shows that the intralaminar Pv, CU and CL nucleus are all implicated in dementia as all three nuclei show increased levels of α-sync and trend level volume reduction in PD dementia (PD-D) compared to PD patients without dementia. When analyses were collapsed across the other Lewy body disease patients who had either PD-D or DLB, the CL nucleus showed the strongest relationship with dementia as more volume loss was evident in this region in the PD-D and DLB groups compared to the non-dementing PD group. The CL nucleus primarily projects to the anterior cingulate and prefrontal cortices (Van der werf, et al., 2002) suggesting that the attention and memory deficits of dementia may be, in part due to pathology in this region.

The intralaminar nuclei have also been implicated in Alzheimer's disease. LB pathology burden progressively affects the intralaminar nuclei while wider cortical pathology is still evolving and a significant linear relationship exists between the degree of LB burden and cortical stage of degeneration (Rub, Del Tredici, Del Turco, et al., 2002).

In vivo techniques have also identified select thalamic nuclei loss in dementia. In PD dementia for example, whole brain voxel based morphometry (VBM) identified significant changes that co-varied with cognitive decline in the pulvinar (Pu) nucleus. In PD-D patients there was also significant grey matter loss in frontal, limbic, parietal and temporal lobes bilaterally compared to non-dementing PD patients but the only subcortical region implicated was the Pu which had significantly reduced grey matter density on the

right side. Robust statistical analyses were conducted on this sample and the reduction remained significant after inclusion of age, sex and disease duration covariates (Beyer, Larsen, et al., 2007).

In Alzheimer's disease, diffusion tensor imaging (DTI) techniques have identified significant cellular disruption in the limbic AP and association MDn nuclei (Chen, Kang, Hu, Hu, & Zou, 2007). The dementia cohort in this instance was an AD group who also had cerebrovascular lesions (ADv). Both limbic nuclei had disrupted cellular integrity – shown by an increase in mean diffusivity (MD), relative to AD patients without lesions. It is impossible to determine whether this increase was due to higher levels of cognitive dysfunction in the ADv group however as only the MMSE was used to evaluate cognitive status of patient groups. Also, as the relationship between MMSE and brain changes was restricted to changes in only the hippocampus, no insight into the thalamic influence on cognition can be provided.

8.3 The identification of thalamic nuclei

As cell size, density and the distribution of cells varies considerably between nuclei, the differentiation of individual thalamic regions is easily achieved under a microscope (Henderson, et al., 2000b). Histological studies are limited to cross-sectional design as these characteristics are only able to be visualised post-mortem (Vernon, et al., 2010) and the *in vivo* visualisation of individual thalamic components has historically been more difficult as structural MR images remain normal in PD (Stoessl, 2011). Although not easily visualised (Magnotta, Gold, Andreasen, Ehrhardt, & Yuh, 2000), it is possible to identify some of the larger thalamic nuclei by mapping the intensity changes between T1 and T2 relaxation times in thalamic regions. The intensity difference is assumed to be reflective of the variations in fibre size, density and myelination which comprise each nucleus (Deoni, Josseau, Rutt, & Peters 2005) but this method does not allow for quantification of cellular changes between regions.

Recently, nuclear imaging has advanced and the longitudinal examination of thalamic nuclei changes in neurodegenerative disease may be possible. New methodology must first be validated however and we seek to do this using a cross-sectional design here where the between group changes are assumed to represent disease progression and allow for the identification of areas that are most likely to degenerate through the course of disease.

Diffusion tensor imaging is a modern method of nuclear medicine that is sensitive to the microstructural architecture of cells and provides measures of water distribution thought to be reflective of underlying cellular structure and integrity (Watts, 2008). DTI methods may be able to detect the changes that occur as a result of neuro-pathology, as these inclusions typically lead to neuronal cell loss and sometimes volume loss in the infiltrated regions (Henderson, et al., 2000a). DTI also allows for the non-invasive *in vivo* visualisation of regional structures and can be used to differentiate between cellular aggregations that are assumed to represent individual thalamic nuclei. The segmentation of thalamic nuclei is achieved through the grouping of similarly orientated axonal fibre tracts that traverse the thalamic region as they travel throughout the cortex, inter connecting multiple subcortical and cortical regions. For the fibres that originate in the thalamus, the principal orientation of every fibre will vary depending on the cortical region of primary connectivity (Niemann, Mennicken, Jeanmonod, & Morel, 2000). Within a nucleus, neuronal axons or fibres will primarily project to the same cortical region and thus will tend to orientate in the same direction (*Figure 8-2*). The segmentation of nuclei therefore, is achieved through the grouping of fibres of the same principal direction by way of algorithms which examine the diffusion images.

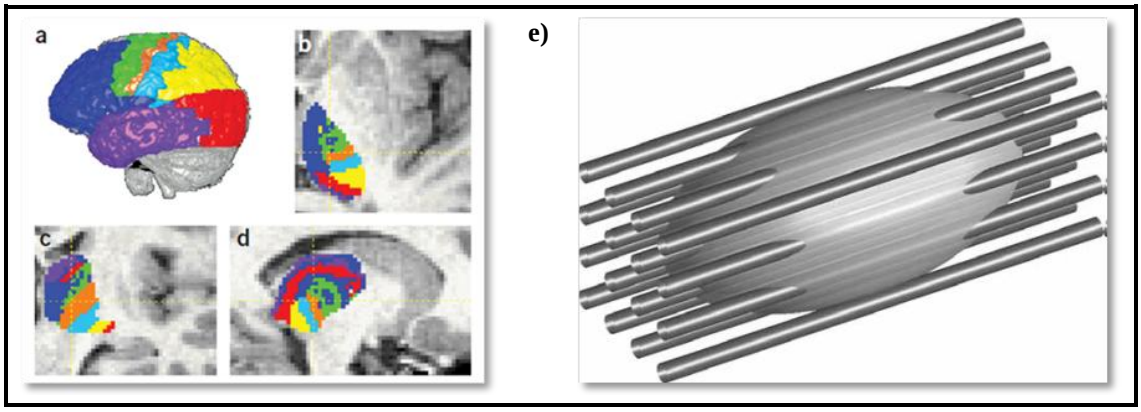


Figure 8-2: Identifying thalamic nuclei using diffusion tensor imaging
Diffusion (represented by an ellipsoid, **e**) is strongest in the direction of prominent fibre orientation, Image from Watts, (2008). Thalamic nuclei (**b, c, d**) have primary connectivity with distinct areas of the cortex (**a**) dictating that fibres within each nuclei will orientate in a similar direction. Image from Behrens, et al., (2003). Fibres of similar orientation can thus be grouped into clusters of thalamic nuclei.

Successful segmentation of thalamic nuclei has been achieved in young, healthy control subjects (Wiegell, et al., 2003) but this method has not yet been validated in a disease sample.

8.4 Summary

The thalamus is topographically organised and each region preferentially connects to a distinct area of the cortex. The thalamic nuclei are thus involved in cognitive processes specific to these regions and these regions are known to be affected in PD, indicating that thalamic nuclei are likely to also be affected. Indeed, histological studies indicate that thalamic nuclei are differentially targeted by Lewy body pathology in PD and this corresponds to neuronal loss. Measurement of cell loss in thalamic nuclei *in vivo* using advanced nuclear imaging techniques could be a sensitive measure of thalamic nuclei integrity. Given the specificity of each region, degeneration of a specific nucleus is expected to correspond to decline in a related domain of cognition. Integrity measures of thalamic nuclei could therefore provide neurocorrelates of cognitive dysfunction across the spectrum of PD. Executive function deficits are particularly relevant, as this is an area of frontal dysfunction that is often the first cognitive symptom a PD patient will present and is one of the strongest contributing factors to later dementia development (Aarsland, et al., 2003).

The intralaminar CM/Pf complex is the only region of the thalamus that has been identified as showing volume loss in PD using MR imaging to date (McKeown, et al., 2008). Although the more sensitive DT imaging technique has previously been applied in PD, this has only identified cellular disruption in the mediodorsal region of the thalamus in PD patients with depression compared to those without (Li, et al., 2010). As yet, imaging methodology has not been applied in a disease sample to all thalamic nuclei and compared to a healthy control group. This study will therefore address a significant gap in the literature and examine the whole thalamic region, aggregated into specific nuclei regions in order to determine the value of diffusion tensor imaging as a neurocorrelate of cognitive dysfunction in Parkinson's disease.

8.5 Hypothesis

- That individual regions of the thalamus will be differentially involved in Parkinson's disease and reflect cognitive dysfunction

8.6 Method

8.6.1 Participants

The identification of the thalamic nuclei depended on judgments of size and location so in instances where the *k*-means clustering procedure produced unusually large clusters or when more than one nucleus (such as the VA and VL) was included in a single cluster this resulted in the exclusion of that subject. Eight patients (PD-N = 5, PD-MCI = 1, PD-D = 2) and one control participant from the original sample (*Section 6.7.1*) were excluded. The final sample of participants for which thalamic nuclei were defined included 51 PD-N, 18 PD-MCI, 15 PD-D patients and 24 control participants. Exclusion of these participants did not result in any major discrepancy of clinical or cognitive characteristics between these groups and those in the previous chapters.

8.6.2 K-means Clustering

The thalamic nuclei were clustered separately for each hemisphere using a *k*-means clustering algorithm which was created specifically for this study. The algorithm was based on an implementation by Wiegell et al., (2003) and was further developed using the specific study parameters for this research by Dr. Tracy Melzer and Dr. Richard Watts of the New Zealand Brain Research Institute. The algorithm requires the definition of four specifications prior to implementation: number of clusters; initialisation of the cluster centroids; distance metric; and a convergence criterion and results in the creation of several clusters of similar voxels. Each cluster is then able to be designated a primary label based on its size, location in the thalamus and approximation to adjacent clusters. While recognising that each cluster is only an approximation of individual thalamic nuclei, they are henceforth labelled by their atlas name.

8.6.2.1 Number of Clusters

To determine the optimal number of clusters to be created for each thalamus the *k*-means clustering algorithm was applied to one image several times using a number of different clusters per hemisphere (n = 14, n = 16, n = 18, n = 20, n = 22, n = 24). The trade-off between too few and too many clusters was optimised at 20 clusters (*Figure 8-3*) as similarly orientated nuclei such as the ventral nuclei combined to form one larger nucleus when *k*-means was set to find less than 20 clusters and small and meaningless clusters were created when the algorithm was set to find any more than 20 clusters.

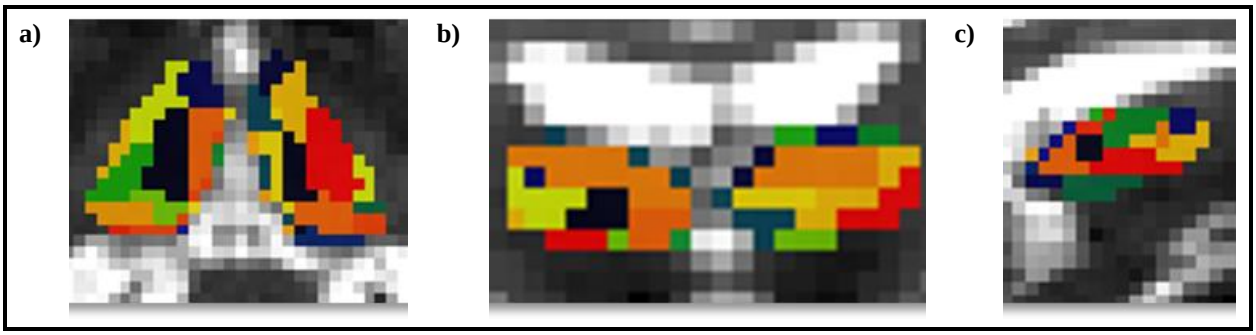


Figure 8-3: Segmented nuclei after the *k*-means clustering procedure
MNI co-ordinates are given in parentheses. The **a)** saggital (Z: 26), **b)** coronal (Y: 63) and **c)** horizontal (X: 68) sections of left and right thalamus (in radiological space) enable simultaneous visualisation of thalamic clusters.

8.6.2.2 Initialisation of the cluster centroids

The centroid is defined as the centre of the nucleus and is first generated based solely on spatial location and is thus uniformly distributed. The first centroid was simply placed at the centre of the thalamus with each subsequent centroid then placed at a maximum distance from all other centroids. The first centroid, for example would be located in the centre of the thalamus, with the second placed on the furtherest voxel from this location. The third centroid is then placed on the voxel furtherest from both the first and second centroid and so forth. This process continues until all centroids are allocated a spatial location.

8.6.2.3 Distance Metric

The distance between voxels was defined using both spatial and tensor parameters to ensure the relationship between distance and direction was optimised. Initial centroids were regenerated so similarly orientated voxels at a maximum distance from all others were combined to form a cluster. For example, if the centroid 1 and the centroid 13 were similarly orientated, they would be combined into one centroid. Specifically, the position-diffusion tensor distance E_{jk} between a voxel *j* and a centroid *k* was taken as a linear combination of the voxel position distance and the diffusion tensor distance, i.e.:

$$\sum_{jk} = \left\| x_j - \bar{x}_k \right\| w_k + y \left\| D_j - \bar{D}_k \right\|$$

where χ_j is the location of voxel $_j$, $\bar{x}_k$ is the mean voxel location for cluster *k*, W_k is the covariance matrix for the voxels in cluster *k*, *y* is a weighting factor to control the trade-off between the diffusion tensor distance and the voxel distance, D_j is the diffusion tensor for voxel *j*, and $\bar{D}_k$ is the mean diffusion tensor for cluster *k*. (Bishop, 1997; Hartigan & Wong, 1979; Wiegell, et al., 2003).

8.6.2.4 Convergence Criterion

The clustering procedure continued through as many iterations as necessary until no voxels within a cluster were changed. There was no restraint on the number of possible iterations although the process was manually disrupted in the event there were more than 1000 iterations, as this indicated the algorithm had become circular. In the majority of cases 20-50 iterations were required before the clusters were defined.

8.6.3 Nuclei Labelling

Following multiple anatomical guidelines (Duvernoy, 1991; Jones, 2007b; Morel, Magnin, & Jeanmonod, 1997) the 20 clusters were assigned to 9 nuclei (MDn, LP, Pu, AP, LD, VA, VL, VP and CM/Pf). Individual nuclei were labelled manually by one observer (N.B.) after reference to all three anatomical planes and consideration of location, size and the proximity to other nuclei. Typically, multiple clusters made up structures such as the mediodorsal nucleus (*Figure 8-4 b*) which has several subdivisions while the lateral nuclei were comprised of one or two clusters (*Figure 8-4 c*).

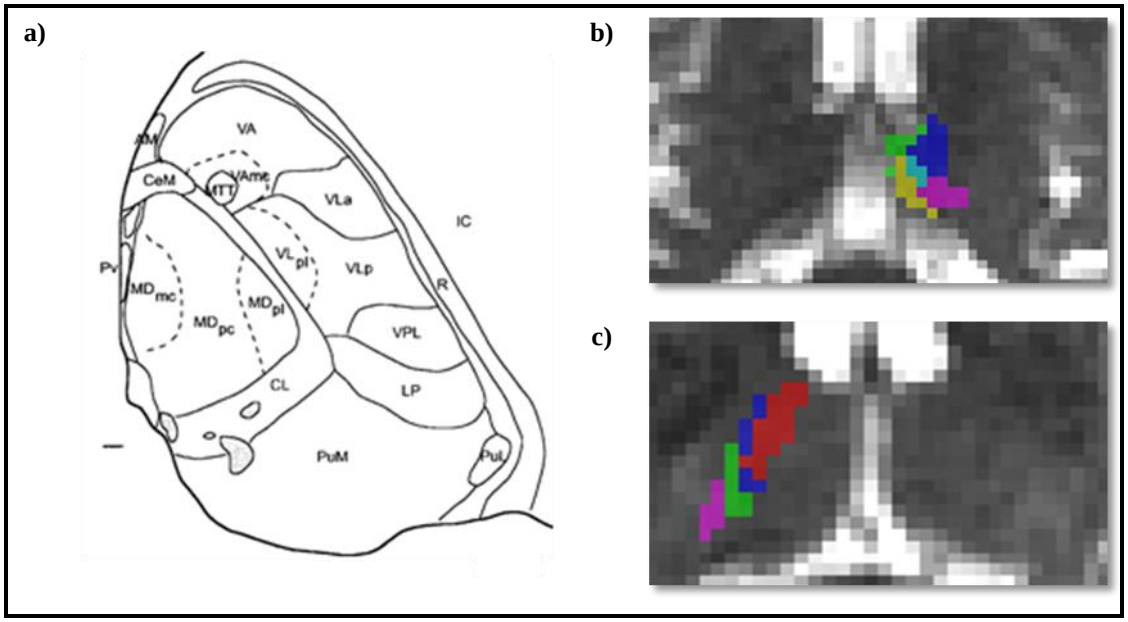
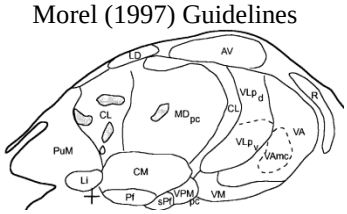
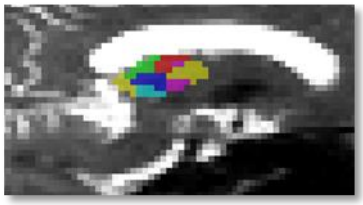
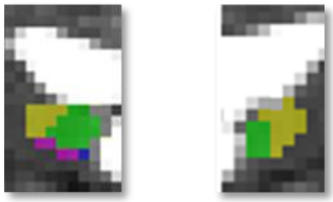
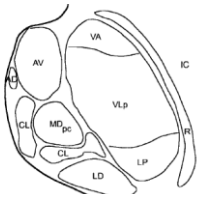
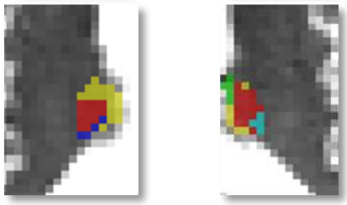
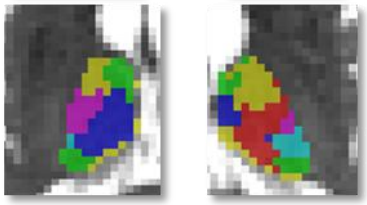
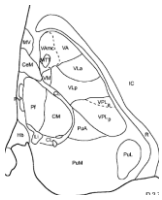
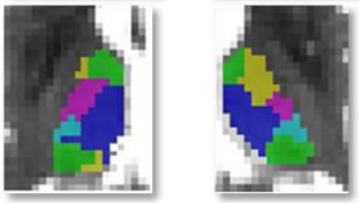
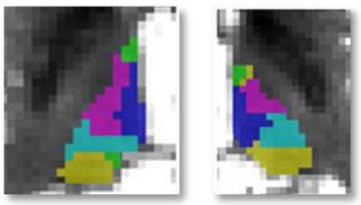
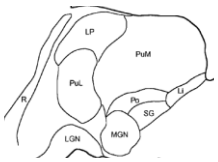


Figure 8-4: Combining clusters to form nuclei
The clusters can be visually inspected and combined to form assumed sub-divisions of the thalamus according to anatomical guidelines, (Morel, et al., 1997) **(a).** In the mediodorsal sub-division **(b)** several clusters are combined: Magnocellular (MD_{mc}) - yellow; parvocellular (MD_{pc}) - green, light blue, purple and paralamellar (MD_{pl}) – dark blue. In the lateral sub-divisions **(c)** only one or two clusters are combined: VA - red; VL - blue, green and VP - purple. All panels are presented in the axial plane.

Table 8-1: Aggregated thalamic clusters that approximate anatomical guidelines for thalamic nuclei

Section and nuclei	Morel (1997) Guidelines	Identified Nuclei
Saggital MDn (red) LP (green) Pu (yellow) CM/Pf (blue) VA (yellow) VL (purple) VP (blue)		
Coronal AP (green) VA (yellow) VL (purple)		
Horizontal – Dorsal sections AP (green) LD (yellow - medial) MDn (red) CM/PF (blue) VP (light blue)		
LP (green) Pu (yellow)		
Horizontal - Ventral section AP (green) VA (yellow) VL (purple) VP (blue) LP (green) Pu (yellow) CM/Pf (blue)		
		
Coronal LP (green) Pu (yellow) CM/Pf (blue)		

The view that the nuclei is best visualised in is presented. Images are in MNI standard space and are referred to using radiological convention, where the left side of the image is the right side of the brain.

The anatomical guidelines used to identify nuclei are shown in *Figure 8-4*, with complete sections presented in *Appendix 12.1*. Cluster aggregations were always viewed simultaneously in three planes. The primary view was dependent on which nucleus was to be defined. The LD, MDn and CM/Pf were identified first, and are best visualised in the

saggital plane. The AP is best distinguished from the VA using the coronal plane, while the lateral nuclei are best identified using horizontal sections, beginning in the most dorsal slice and following continuous slices until the most ventral slice is reached. Finally, the LP and Pu are easily identified in the coronal section, where the LP is immediately dorsal to the Pu, although these structures are also easily identified in the horizontal section where the LP is immediately anterior to the Pu.

8.6.4 Statistical Analysis

Between group differences were examined using a between and within groups analysis of variance (RM ANOVA) where the dependent variable was the measure of an individual thalamic nucleus (either volume, FA or MD), the independent variable was group (healthy controls, PD-N, PD-MCI and PD-D) and the within groups factor was hemisphere (left and right). Only significant interactions are reported. In the case of a significant group effect Newman-Keuls post-hoc comparisons were conducted. In the case of a group x hemisphere interaction, follow up one-way ANOVA's were conducted to examine the group effect independently of the hemisphere effect. Kruskal-Wallis ANOVA was employed when non-parametric analyses were required. To control for the confounding variables: age, education and level of depressive symptoms these were added to the statistical model in an analysis of covariance (ANCOVA). To focus on the changes specific to Parkinson's disease the control subjects were also excluded and the clinical covariates (disease duration and UPDRS III) additionally included in a similar ANCOVA model.

In the first instance the relationship between nuclei and cognition was examined using Pearson simple correlations to determine if the relationship between the two was linear, as required to meet the assumptions for multiple regression. All measures (vol, FA, MD) of all nuclei were included. Subsequently, those nuclei showing a significant association with cognition were included in a series of backward elimination stepwise regressions where two models were used following the methodology of Stewart, et al., (2009). To examine the proportion of unique variance in each of the cognitive domains (attention, learning and memory, executive function, visuospatial function) attributable to thalamic nuclei measures, a block of thalamic nuclei were entered as predictor variables. The unique proportion of variance attributable to each thalamic nucleus was determined by proceeding through several backward elimination steps where the nucleus that contributed the least variance was removed, followed by the next least variance and so forth until the

final model only included those nuclei that contributed a significant proportion of the variance in that cognitive domain. A second block of predictors was then entered for each model to examine the effect of covariates. Covariates (age, education, disease duration and depression) were entered alongside all nuclei that were originally entered in Model One. The change in the R^2 coefficient from the first block of predictors to the second block represented the percentage of unique variance in a given cognitive measure attributable to that combination of nuclei. The individual contribution of each nucleus was also determined from each final model using the Beta values. The bilateral, as opposed to separate left and right measures were used for these analyses in order to prevent violation of the multi-colinearity assumption.

Finally, the predictive validity of each nucleus ROI was examined using receiver operating characteristics curves (ROC). Several pairwise comparisons were conducted for levels of cognitive impairment where each group was compared to both the control group and the PD-N group. AUC statistics and the corresponding sensitivity levels are reported at least 80% specificity.

8.7 Results

8.7.1 Participant information

The demographic and clinical measures of participants for whom clustering of thalamic nuclei were determined are shown in (*Table 8-2*). This sample is similar to that of the previous chapter (*Section 6.6.1*), only eight patients (PD-N = 5, PD-MCI = 1, PD-D = 2) and one control participant have been excluded from the following analyses. As before, the characteristics of the groups remained the same in terms of education, depressive symptoms, and the distribution of sex and handedness between groups and all four groups were well matched on these variables. In terms of age, the PD-N group remained younger than the PD-D group but was now well matched to the PD-MCI group. As before, all clinical variables showed a steady increase from PD-N to PD-MCI to PD-D.

Global cognitive, cognitive domain scores and individual neuropsychological test scores are shown in *Table 8-3*. The overall distribution of results mirrored that found previously. The control and PD-N groups had significantly superior performance on all cognitive measures except the digit span and RCFT immediate recall and the PD-MCI group on all measures except the map search when compared to the PD-D group. Neuropsychological performance of the control and the PD-N group was generally above the standardised mean score for most tests and the two groups comparable on all tests. The aggregated domain scores were all significantly higher in the control group in all areas of cognition except in the area of visuospatial/visuoperceptual function. Cognition in the mild cognitive impairment group was generally of an intermediate nature and mostly significantly different to both the PD-D and the PD-N group.

Table 8-2: Mean (SD) of demographic and clinical data for all groups

	Control (n = 24)	PD-N (n = 51)	PD-MCI (n =18)	PD-D (n = 15)	Statistic	p	Adjacent pairwise comparisons
Demographic							
Age	67.42 (9.8)	64.61 (8.5)	70.50 (8.6)	73.20 (7.1)	F= 4.8	<0.01	C = N = MCI= D
Education	13.88 (2.8)	13.59 (3.1)	12.56 (2.9)	12.60 (2.3)	F= 1.2	=0.33	C = N = MCI = D
Depression (GDS) [median (range)]	0.04 (0.2)	1.10 (2.1)	1.33 (2.9)	3.20 (3.3)	H= 14.4	<0.01	C = N = MCI = D
Sex (M/F)	16/8	33/18	12/6	13/2	χ^2 = 2.7	=0.44	
Handedness (R/L)	19/4	49/2	16/2	14/1	χ^2 = 4.0	=0.26	
Premorbid IQ (WTAR)	118.3 (9.8)	114.5 (8.5)	107.0 (12.8)	108.5 (9.4)	H= 15.6	=0.01	C > N > MCI = D
Clinical							
Hoehn + Yahr [median (range)]		2.00 (1 - 3)	2.50 (1.5- 4)	4.0 (2 - 4)	F= 22.4	>0.01	N > MCI > D
UPDRS III (max=88)		24.00 (0 -70)	30.50 (9 - 69)	52 (18 - 81) *n=14*	F= 20.4	>0.01	N > MCI > D
Disease Duration		4.11 (3.5)	7.86 (5.2)	12.68 (9.1)	H= 18.3	>0.01	N > MCI > D

Although 9 subjects were lost in this subset due to imaging constraints the same general pattern emerges for demographic and clinical variables. The groups are well matched in terms of age, education and sex and handedness distributions. As expected, there is progressive worsening of clinical motor scores from the PD-N group to the PD-MCI group with the worst scores evident in the PD-D group. **GDS:** Geriatric depression scale; **WTAR:** Weschler test of adult reading; **UPDRS III:** Unified Parkinson's disease rating scale. All participants completed all tests unless otherwise indicated by n value. **F**: ANOVA F ratio; **H:** Kruskall-Wallis statistic; **χ^2:** chi-square analysis.

Table 8-3: Mean (SD) of average cognitive domain and individual neuropsychological test Z-scores for all participants

	Control (n = 24)	PD-N (n = 51)	PD-MCI (n = 18)	PD-D (n = 15)	Statistic	p	Adjacent pairwise comparisons
Global Cognitive							
MMSE (max=30)	28.71 (1.5)	28.47 (1.5)	26.22 (2.4)	23.27 (3.3)	H= 43.3	<0.01	C = N > MCI > D
MoCA (max=30)	27.75 (1.5) *n=48*	26.77 (2.2)	22.56 (2.4)	17.07 (3.6)	H= 64.0	<0.01	C = N > MCI > D
Attention and working memory+	0.39 (0.4)	-0.02 (0.5)	-0.88 (0.5)	-1.84 (0.5)	F= 83.5	<.001	C > N > MCI > D
Digits Forward/Backwards	1.1 (1.1)	0.4 (0.8)	-0.0 (0.9)	-0.7 (1.0)	F= 11.9	<.001	C > N = MCI > D
Digit Ordering	-0.4 (0.9)	-0.5 (1.0)	-1.7 (0.7)	-2.2 (0.6)	F= 21.3	<.001	C = N > MCI = D
Map Search	0.7 (1.0) *n=46*	-0.2 (0.9)	-1.7 (0.8)	-2.2 (0.8)	F= 43.3	<.001	C > N > MCI = D
Stroop Colour Trial	0.2 (0.8) *n=50*	0.1 (0.9)	-0.5 (0.8)	-1.9 (0.9)	F= 23.1	<.001	C = N > MCI > D
Stroop Word Trial	0.4 (0.6)*n=50*	0.1 (0.6)	-0.5 (0.8)	-1.5 (1.2)	H= 32.3	<.001	C = N > MCI > D
Trail Making Test A	0.3 (0.9)	-0.1 (0.7) *n=17*	-0.8 (1.0)	-2.6 (0.5)	F= 53.3	<.001	C = N > MCI > D
Executive Function+	0.88 (0.5)	0.43 (0.6)	-0.73 (0.8)	-1.98 (0.5)	F= 84.1	<.001	C > N > MCI > D
Letter Fluency	1.1 (1.2)	0.8 (1.2)	-0.2 (1.1)	-1.4 (0.9)	F= 18.8	<.001	C = N > MCI > D
Action Fluency	0.7 (1.0)	0.3 (1.3)	-0.9 (1.0)	-1.6 (0.6)	H= 40.5	<.001	C = N > MCI > D
Category Fluency	1.2 (0.9)	0.9 (1.2)	-0.4 (1.0)	-1.4 (0.8)	F= 27.8	<.001	C = N > MCI > D
Category Switching	1.1 (0.9)	0.2 (0.9)	-0.7 (0.8)	-2.0 (0.9)	F= 46.0	<.001	C > N > MCI > D
Trail Making Test B	0.6 (0.5)	-0.1 (0.9) *n=17*	-0.9 (1.2)	-2.9 (0.1)	H= 49.8	<.001	C > N > MCI > D
Stroop Interference	0.6 (0.5) *n=50*	0.4 (0.7)	-1.2 (1.5)	-2.5 (0.8)	H= 49.7	<.001	C = N > MCI > D
Learning and Memory+	0.92 (0.8)	0.35 (0.8)	-0.74 (0.5)	-1.60 (0.7)	F= 46.1	<.001	C > N > MCI > D
CVLT Free Recall	1.0 (0.9)	0.5 (0.9)	-0.9 (0.9)	-1.8 (1.0)	F= 39.6	<.001	C = N > MCI > D
CVLT Short Delay (30s)	1.1 (1.3)	0.4 (1.1)	-0.7 (1.0)	-1.7 (0.8)	H= 43.4	<.001	C = N > MCI > D
CVLT Long Delay (10min)	0.8 (0.8)	0.4 (0.9)	-0.5 (0.8)	-0.9 (0.7)	F= 18.6	<.001	C = N > MCI = D
RCFT Immediate (3 min)	1.0 (1.3)	0.2 (1.4)	-0.6 (1.3)	-1.8 (0.9)	F= 15.2	<.001	C > N = MCI > D
RCFT Delayed (30 min)	0.8 (1.5) *n=22*	-0.3 (1.5) *n=24*	-1.2 (1.2) *n=13*	-1.9 (1.1)	F= 12.1	<.001	C > N > MCI > D
Visuospatial/Perception function+	0.58 (0.6)	0.47 (0.4)	-0.40 (0.6)	-1.26 (0.8)	H= 53.7	<.001	C = N > MCI > D
RCFT Copy	0.3 (0.9)	0.1 (0.8)	-1.0 (1.3)	-2.1 (1.3)	F= 24.3	<.001	C = N > MCI > D
JOL	0.7 (0.6)	0.6 (0.5)	-0.2 (0.9)	-0.8 (1.1)	F= 21.6	<.001	C = N > MCI > D
VOSP Fragmented Letters	0.8 (0.8) *n=23*	0.7 (0.5)	-0.0 (1.1)	-0.9 (1.1)	H= 25.4	<.001	C = N > MCI > D
Global Z+	0.69 (0.4)	0.32 (0.4)	-0.69 (0.4)	-1.67 (0.5)	F=138.9	<.001	C > N > MCI > D

As expected worse performance was evident in the PD-D group. In most cases the PD-N group performed at a level similar to that of the control group while the PD-MCI group was consistently performing at an intermediary level. + age adjusted Z score. Global Z score is an aggregate mean score of the mean value in each cognitive domain. **MMSE:** Mini mental state examination; **MoCA:** Montreal cognitive assessment. **CVLT:** California verbal learning test; **RCFT:** Rey complex figure test; **JOL:** Judgement of line orientation; **VOSP:** Visual object space perception battery. All participants completed all tests unless otherwise indicated by *n*.

8.7.2 Volumetric changes of thalamic nuclei

Nucleus volume was first computed relative to the individuals intracranial volume (nucleus divided by ICV for each subject) to adjust for head size. Between – within ANOVA examined group differences for each nucleus with co-variates age, education and depression and then disease duration and UPDRS III additionally added in subsequent models. Mean values of thalamic nuclei ROI's and ICV are shown in *Table 8-4.* There were no group differences of mean ICV and thalamic nuclei volumes relative to ICV except in the AP nucleus. This may be an unreliable finding however as Newman-Keulis post-hoc results show the effect to be due to trend level volume reduction in the PD-N group only. The group effect remained when the demographic covariates (age, education and depression) were added to the model [$F(3,101) = 3.73$, $p = 0.01$] but not [$F(2,76) = 1.39$, $p = 0.26$] after inclusion of the clinical covariates (disease duration and UPDRS III). Regions of the association (MDn), limbic (LD) motor (VA) and non-specific CM/Pf thalamic nuclei also showed a trend for volume loss, with the PD-D group consistently showing the lowest volumes for all comparisons. A main effect of hemisphere was evident in the left > right direction for the MDn and LP nuclei but this was consistent for all groups; there were no group x hemisphere interactions.

Table 8-4: Comparison of nuclei volumes (corrected for ICV) between PD groups and control subjects

	Control (n = 24)	PD-N (n = 51)	PD-MCI (n = 18)	PD-D (n = 15)	Group Effect		Hemisphere Effect	
					F	p	F	p
Association Nuclei								
MDn	1.86 (0.4)	1.82 (0.3)	1.83 (0.4)	1.70 (0.3)	0.47	0.70	13.01	<0.001
Left	1.0 (0.3)	1.0 (0.2)	1.0 (0.2)	0.9 (0.2)				
Right	0.8 (0.2)	0.9 (0.2)	0.9 (0.3)	0.8 (0.2)				
LP	0.55 (0.1)	0.57 (0.1)	0.53 (0.2)	0.55 (0.2)	0.43	0.73	4.51	0.04
Left	0.3 (0.1)	0.3 (0.1)	0.3 (0.1)	0.3 (0.1)				
Right	0.3 (0.1)	0.3 (0.1)	0.2 (0.1)	0.3 (0.2)				
Pu	0.93 (0.2)	0.94 (0.2)	0.99 (0.2)	0.94 (0.2)	0.37	0.78	0.82	0.37
Left	0.5 (0.1)	0.5 (0.1)	0.5 (0.1)	0.5 (0.1)				
Right	0.4 (0.1)	0.5 (0.1)	0.5 (0.2)	0.5 (0.1)				
Limbic Nuclei								
AP	0.51 (0.1)	0.41 (0.2)	0.48 (0.2)	0.50 (0.1)	3.12	0.03	1.43	0.24
Left	0.3 (0.1)	0.2 (0.1)	0.2 (0.1)	0.2 (0.1)				
Right	0.3 (0.1)	0.2 (0.1)	0.3 (0.1)	0.3 (0.1)				
LD	0.44 (0.2)	0.43 (0.2)	0.44 (0.1)	0.42 (0.1)	0.09	0.96	2.13	0.15
Left	0.2 (0.1)	0.2 (0.1)	0.2 (0.0)	0.2 (0.1)				
Right	0.2 (0.1)	0.2 (0.1)	0.3 (0.1)	0.2 (0.1)				
Sensory Nucleus								
VP	0.74 (0.2)	0.79 (0.3)	0.83 (0.2)	0.76 (0.24)	0.51	0.68	0.64	0.43
Left	0.4 (0.2)	0.4 (0.2)	0.4 (0.2)	0.4 (0.2)				
Right	0.4 (0.2)	0.4 (0.2)	0.4 (0.1)	0.4 (0.2)				
Motor Nuclei								
VA	0.82 (0.2)	0.83 (0.3)	0.74 (0.2)	0.73 (0.3)	0.95	0.42	1.16	0.28
Left	0.4 (0.2)	0.5 (0.2)	0.4 (0.2)	0.4 (0.2)				
Right	0.4 (0.2)	0.4 (0.2)	0.4 (0.1)	0.4 (0.2)				
VL	0.74 (0.3)	0.76 (0.2)	0.79 (0.2)	0.78 (0.2)	0.22	0.88	0.03	0.88
Left	0.4 (0.2)	0.4 (0.1)	0.4 (0.1)	0.4 (0.1)				
Right	0.3 (0.2)	0.4 (0.2)	0.4 (0.2)	0.4 (0.1)				
Non-Specific Nucleus								
CM/Pf	1.72 (0.4)	1.72 (0.4)	1.73 (0.3)	1.58 (0.3)	0.93	0.43	3.40	0.07
Left	0.9 (0.2)	0.9 (0.2)	0.9 (0.3)	0.8 (0.2)				
Right	0.8 (0.3)	0.8 (0.2)	0.8 (0.2)	0.8 (0.2)				
ICV (cm^3)	1495.6 (151.1)	1545.5 (169.1)	1478.0 (100.2)	1608.5 (183.1)	2.42	0.07		

ICV corrected nuclei volumes are reported mean (SD) in mm^3 $x10^{-3}$. Repeated measures ANOVA was used to examine group and hemisphere effect in the first instance (indicated in **bold** face). No follow up analyses were needed as there was no group x hemisphere interaction in any nucleus. The AP nucleus was the only region to show a group effect, there were no significant post-hoc comparisons. The hemisphere effect in the MDn and LP nuclei was in the left > right direction.

8.7.2.1 Relative to thalamic volume

Nucleus volume was also calculated relative to thalamic volume (nucleus in each hemisphere divided by whole thalamic volume of that hemisphere x 100) and expressed as a proportion of total thalamic volume (*Figure 8-5)* to correct for the variation in overall thalamic degeneration between groups. In accordance with anatomical guidelines

(Duvernoy, 1991; Morel, et al., 1997) the largest aggregations were the MDn and CM/Pf nuclei and the smallest the anterior principal and lateral dorsal aggregates. Results were similar when ROI's were corrected for thalamic volume as they were when corrected for ICV, there were again no between group differences of nuclei volume except for the AP nucleus [$F(3,104) = 4.1$, $p < 0.01$]. This effect remained when demographic variables and depressive scores were included in the model [$F(3,101) = 4.2$, $p = 0.04$] but not when the additional clinical covariates were included [$F(2,76) = 1.06$, $p = 0.35$] suggesting significant influence of motor dysfunction on this finding. There was again a significant hemisphere effect (left > right) in the MDn nucleus [$F(1,104) = 6.09$, $p = 0.02$] and no interactions between group and hemisphere.

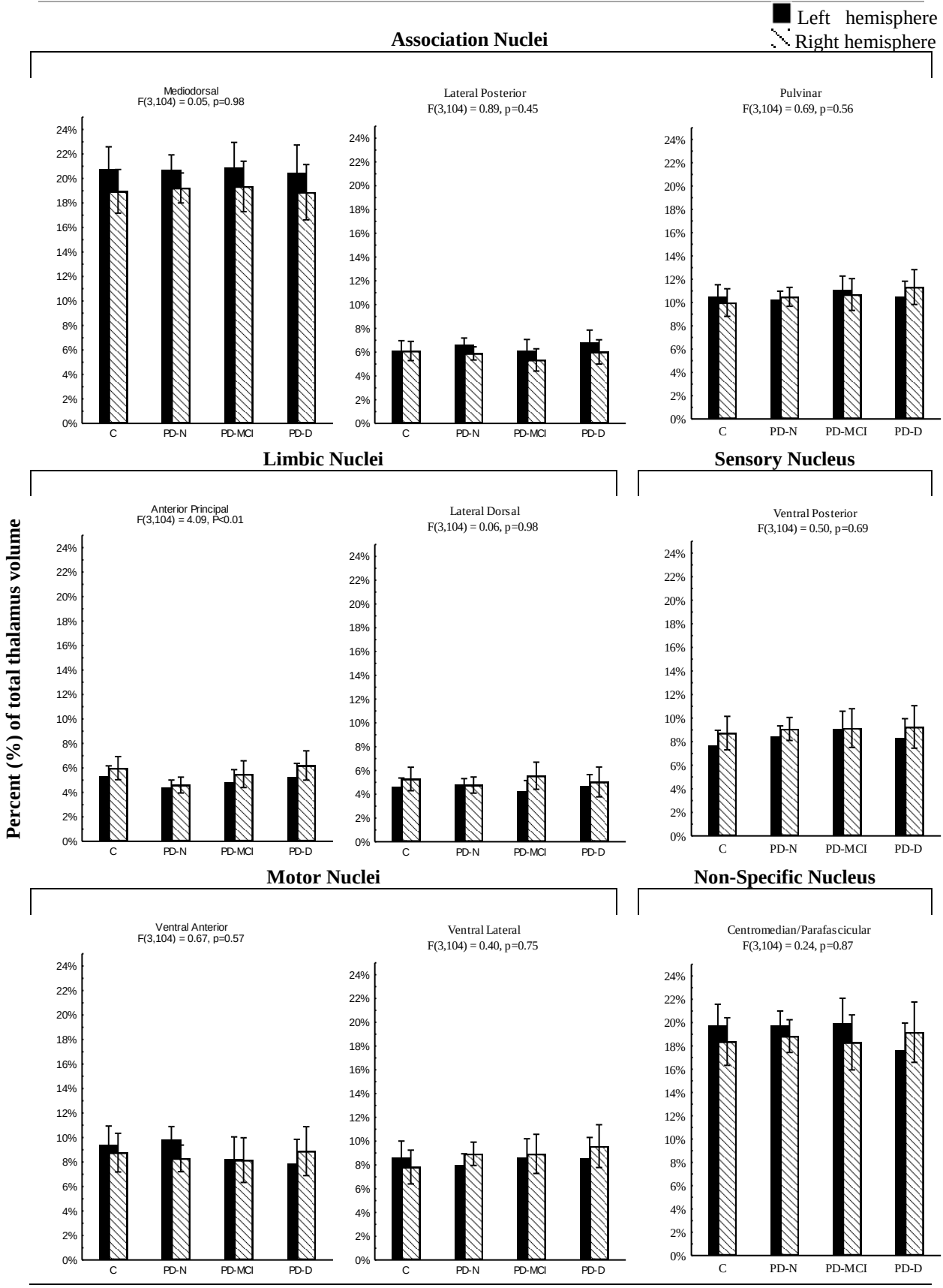


Figure 8-5: Volume of nuclei relative to whole thalamus
Group mean nuclei volume differences are presented for each nucleus. Lines represent standard error bars. Repeated measures ANOVA examined between group differences, results were similar as when thalamus was examined relative to ICV. The AP nucleus was the only region to show a significant effect of group but post-hoc results did not reach significance.

8.7.3 Fractional anisotropy changes of thalamic nuclei

Mean fractional anisotropy values for each thalamic nucleus were compared in the same way as they were for volume except that a weighted average was calculated for each region to account for the variation in size between nuclei. The FA values across thalamic nuclei aggregations for each group are shown in *Figure 8-6*. FA did not differ between groups except in the lateral dorsal nucleus. Post-hoc results showed integrity was significantly reduced in PD-D as well as in PD-MCI compared to the control group and in PD-D compared to PD-N. The group effect and post-hoc comparisons remained when demographic covariates were added to the model [$F(3,101) = 2.95$, $p = 0.04$] but were no longer significant when control subjects were excluded and clinical covariates added [$F(2,76) = 1.76$, $p = 0.18$], suggesting directionality integrity co-varies with motor dysfunction in this area. There was a significant hemisphere effect (left > right) in the AP [$F(1,104) = 13.09$, $p = 0.001$]; VA [$F(1,104) = 20.91$, $p < 0.001$]; VP [$F(1,104) = 19.56$, $p < 0.001$]; and Pu [$F(1,104) = 4.24$, $p = 0.04$] nuclei and an interaction between group and hemisphere in the MDn nucleus [$F(3,104) = 4.80$, $p < 0.01$]. For the MDn nucleus the hemisphere effect (left > right) was only evident in the control group, suggesting that the left hemisphere is sensitive to cognitive dysfunction in PD, irrespective of cognitive status. Follow up ANOVA on the group effect of integrity in the left MDn only showed trend level reduction in FA [$F(3,104) = 2.44$, $p = 0.07$].

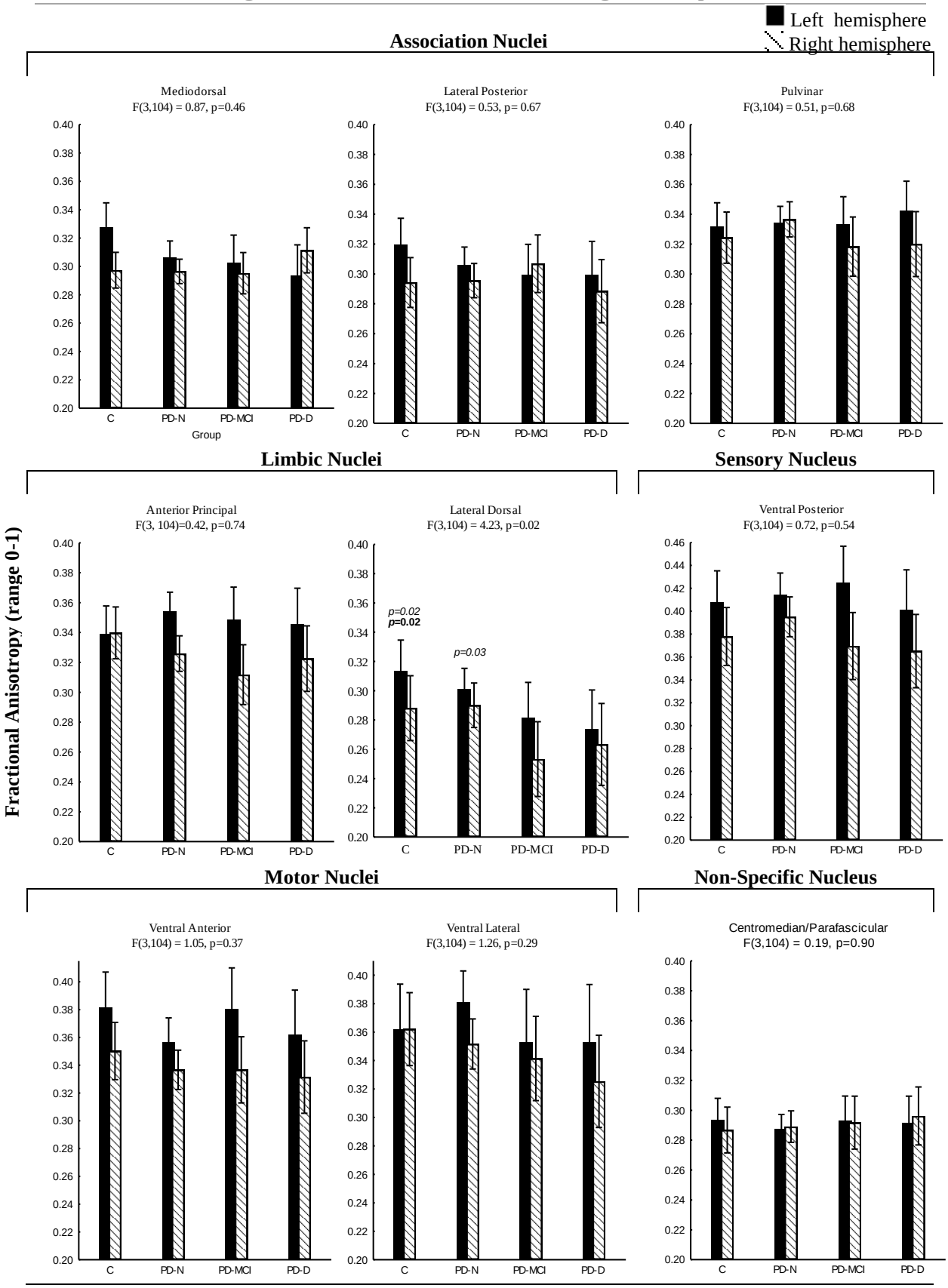


Figure 8-6: FA values of thalamic nuclei
Mean FA values for each ROI, lines represent standard error bars. *p* indicates significant post hoc comparisons between groups (collapsing left and right), *italic* = comparison between dementia group, **bold** = comparison between MCI group. The LD is the only nucleus to show reduction in FA between groups.

8.7.4 Mean diffusivity changes of thalamic nuclei

Mean MD values across the thalamic nuclei clusters are shown in *Figure 8-7* and were also computed using the weighted average formula. In contrast to volume results, there was a significant group effect in all nuclei except the AP. The AP results could again be an unreliable finding due to the significant group x hemisphere interaction [$F(3,104) = 3.36$, $p = 0.02$] which resulted in an effect of group only in the right [$F(3,104) = 5.66$, $p < 0.001$] hemisphere. For all other regions post-hoc results showed diffusivity to be higher in those groups with worse cognitive dysfunction; MD was higher in the PD-D group compared to control subjects and the PD-N group in all regions except the AP; and higher in PD-MCI compared to control subjects in the MDn, LD and Pu and in the LD and MDn compared to PD-N. In contrast to the volume and FA results, there was only an effect of hemisphere in the VP [$F(1,104) = 12.20$, $p < 0.001$] which was in the left > right direction.

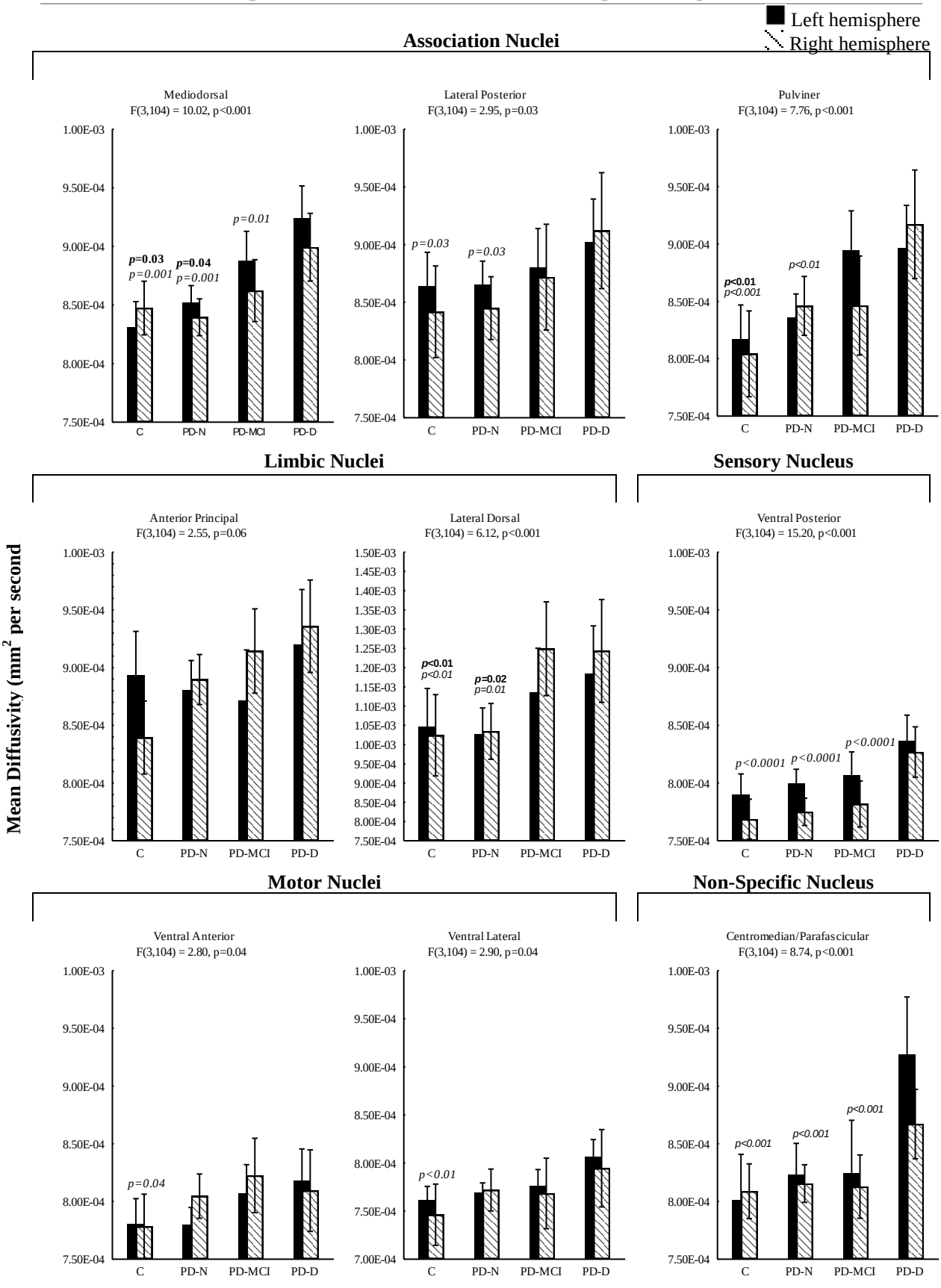


Figure 8-7: MD Values of thalamic nuclei.
Mean MD values for each ROI, lines represent standard error bars. *p* indicates significant post hoc comparisons between groups (collapsing left and right), *italic* = comparison between dementia group, **bold** = comparison between MCI group.

There were few changes once demographic and clinical covariates were accounted for. *Figure 8-8* shows which nuclei are most affected in PD-MCI and PD-D relative to healthy control and PD-N groups with the corresponding group effect for each nucleus presented in *Table 8-5*. In PD-D relative to control subjects all nuclei (including the previously not changed AP nucleus) had significantly higher MD levels. In PD-D relative to PD-N where clinical covariates were also accounted for all nuclei except the AP, VA or VL nuclei had higher MD values. As the VA and VL are motor nuclei and were implicated prior to the inclusion of clinical covariates in the model this demonstrates the adequate control of motor symptoms identified using disease duration and UPDRS III measures. In PD-MCI relative to control subjects only the MDn, LD and Pu continued to show higher diffusivity after inclusion of demographic covariates. The MDn and LD continued to show higher diffusivity relative to PD-N, indicating findings were not due to the symptoms of Parkinson's disease alone. MD measures of nuclei appear to primarily reflect cognitive status. As cognitive decline increases in the PD-D group relative to the PD-MCI group so does diffusivity in the MDn, CM/Pf and VP nuclei. In the cognitively similar PD-N and control groups there were no differences in mean diffusivity for any nuclei.

After the inclusion of demographic covariates there was an additional hemisphere effect [$F(1,101) = 4.17$, $p = 0.04$] in the VL nucleus (left > right). The hemisphere effect in the VP nucleus prior to the inclusion of covariates was no longer significant. In the AP, the group x hemisphere interaction [$F(3,101) = 3.16$, $p = 0.03$] remained due to there again being a significant group effect in the right hemisphere only [$F(3,104) = 5.66$, $p < 0.01$].

The hemisphere effect in the VL nucleus remained after the inclusion of clinical covariates [$F(1,75) = 4.10$, $p = 0.05$] but the group x hemisphere interaction in the AP nucleus was no longer significant.

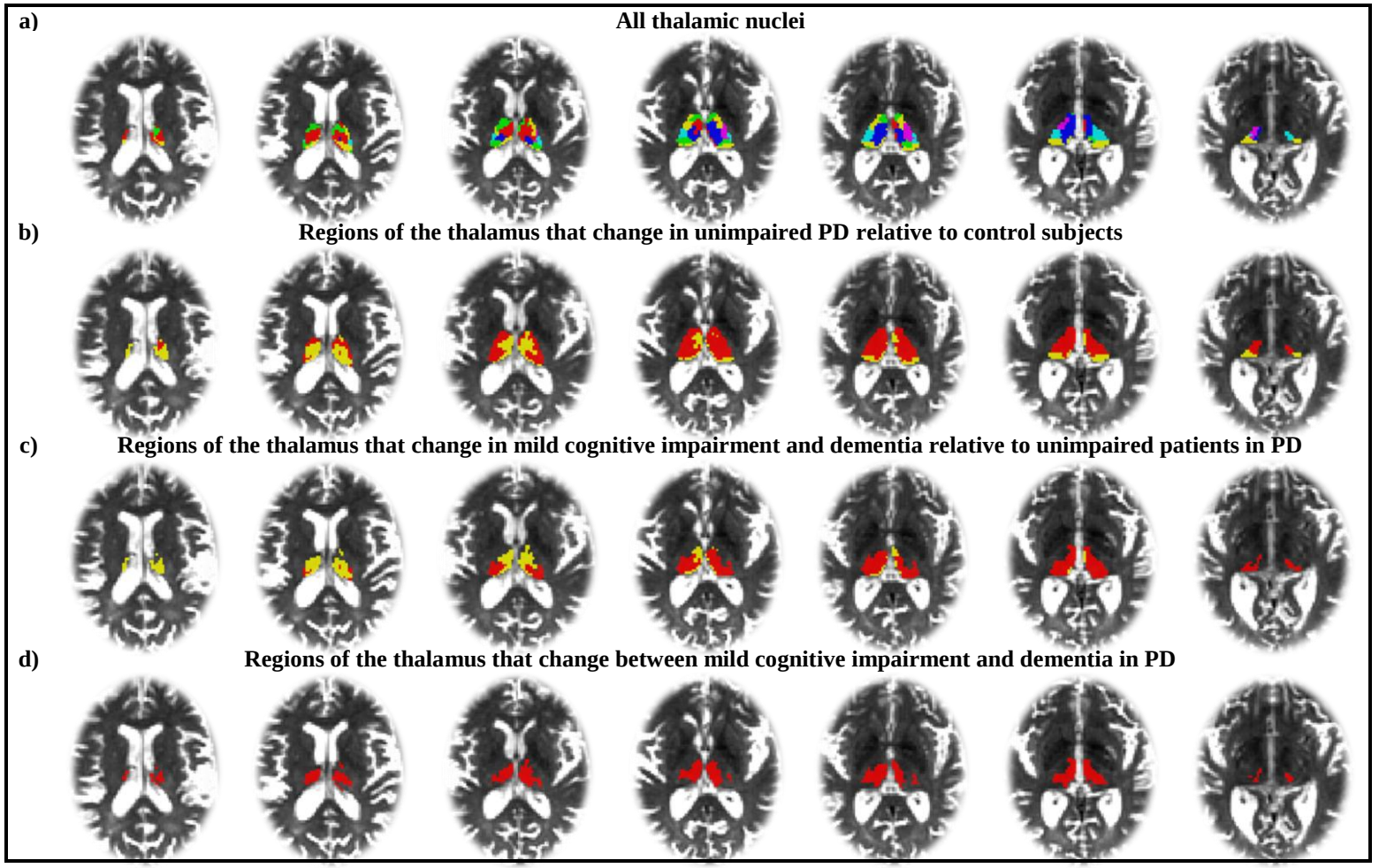


Figure 8-8: The changes in mean diffusivity between groups
Horizontal sections (dorsal – ventral) are presented using radiological convention, the left side of the image corresponds to the right side of the brain. Row **a**) shows all thalamic nuclei where AP is green, MDn red, LD yellow, VP light blue, CM/Pf blue, VA yellow, VL violet. The LP and Pu, visible only in ventral slices are also represented in green and yellow respectively. Row **b**) – **d**) shows regions of change in PD-N (green), PD-MCI (yellow) and PD-D (red) relative to control subjects (**b**), patients without cognitive impairment (**c**) and in PD-D relative to PD-MCI (**d**). Image is the 4D image of a PD-N patient, MNI co-ordinates Z: 30-24.

Table 8-5: Between group comparisons of MD values after the inclusion of covariates

	Covariates					
	Demographic			Clinical		
	Age, education, depression			Disease duration, UPDRS III		
Nuclei	Group Effect			Group Effect		
	F(3,101)	*p*	Adjacent pairwise	F(2,75)	*p*	Adjacent pairwise
Association Nuclei						
MDn	4.87	0.003	C=N<MCI<D	2.67	0.08	N<MCI<D
LP	1.65	0.18	C=N<D	0.51	0.60	N<D
Pu	5.85	0.001	C=N<D	1.40	0.25	N<D
Limbic Nuclei						
AP	1.49	0.22	C<DΔ	0.48	0.62	N=MCI=D
LD	3.99	0.01	C=N<MCI=D	4.24	0.02	N<MCI=D
Sensory Nucleus						
VP	5.15	0.002	C=N=MCI<D	2.46	0.09	N=MCI<D
Motor Nucleus						
VA	1.49	0.22	C<D	0.37	0.69	N=MCI=D
VL	1.65	0.18	C<D	1.35	0.27	N=MCI=D
Non-specific nucleus						
CM/Pf	4.91	0.003	C=N=MCI<D	6.37	0.003	N=MCI<D

The group effect and adjacent pairwise comparisons for each nucleus after inclusion of demographic and clinical covariates is presented. **F:** RM ANCOVA F ratio; ***p:*** *p* value; Δ denotes a significant group x hemisphere interaction; **MDn:** mediodorsal; **LP:** lateral posterior; **Pu:** Pulvinar: **AP:** anterior principal; **LD:** lateral dorsal; **VP:** ventral posterior; **VA:** ventral anterior; **VL:** ventral lateral; **CM/Pf:** centromedian/parafascicular thalamic nuclei; **C:** control participants; **N:** patients with intact cognition; **MCI:** patients with mild cognitive impairment; **D:** patients with dementia.

8.7.5 The association between the integrity of thalamic nuclei and cognition

8.7.5.1 Correlation results

Simple Pearson correlations were used in the first instance to determine which nuclei had an association with cognition so these could be later included in a regression model. Similar to the analyses conducted in *Chapter 6* where the whole thalamus was examined, volume and both diffusion measures of integrity (FA and MD) were examined for their relationship with cognition. Correlations were conducted for the control and patient groups in the manner of Little, et al., (2010) in order to avoid biasing the results due to the fact the patient groups have lower volume and FA and higher MD than control subjects.

Healthy controls

Thalamic mean diffusivity was the measure most sensitive to cognitive changes and had a moderate to strong correlation with the most areas of cognition. The AP was correlated with learning and memory (r = -0.46, p = 0.02), the MDn (r = -0.50, p = 0.01) and VP (r = -0.55, p = 0.01) with executive function, and the VP with attention (r = -0.42, p = 0.04) and the aggregate global score (r = -0.41, p = 0.05). The motor VL motor nuclei was also negatively correlated with learning and memory (r = -0.45, p < 0.03) and global Z score (r = -0.48, p = 0.02).

Thalamic fractional anisotropy was only sensitive to visuospatial/perception and had a strong positive correlation (r = 0.42, p = 0.04) with the VP nucleus.

Only the VP nucleus had a significant association with cognition when volume measures were examined, showing a significant positive correlation with the visuospatial (r = 0.49, p = 0.02) and learning and memory (r = 0.50, p = 0.01) domains as well as with global Z score (r = 0.48, p = 0.02).

Parkinson's disease

Pearson correlation results for the patient group are reported in *Table 8-6*. Mean diffusivity measures of the thalamic nuclei are associated with cognition. The correlation coefficient was generally smaller than that seen in the control group, but there was a moderate relationship between the majority of the cognitive domains and all nuclei except the motor nuclei. All limbic (AP, LD), association (MDn, Pu, LP, VP) and the diffuse non-specific CM/Pf nuclei were associated with the executive function, visuospatial, learning and memory and aggregate global Z score. For attention and working memory only one of the limbic (LD), some of the association (MDn, and Pu), the sensory VP and the CM/Pf nuclei showed a positive relationship with this domain score.

Fractional anisotropy measures were also associated with cognition in the patient group. As in the control subjects, FA of the VP was associated with the visuospatial domain and in addition to this, FA measures of the LD nucleus had a moderate to strong positive relationship with all cognitive domains and global Z score.

In contrast to the control subjects, only the AP nucleus was implicated in cognition using volume measures, showing a moderate negative relationship with learning and memory.

Table 8-6: Relationship between thalamic nuclei measures and cognition

	Attention	Executive Function	Visuospatial	Learning and Memory	Global Z Score
Mean Diffusivity					
Association nuclei					
MDn	-0.35**	-0.38***	-0.38***	-0.44***	-0.44***
LP	-0.17	-0.23*	-0.31**	-0.22*	-0.25*
Pu	-0.34**	-0.30**	-0.28**	0.28**	-0.34**
Limbic nuclei					
AP	-0.20	-0.24**	-0.22*	-0.23**	-0.26**
LD	-0.38**	-0.28**	-0.39***	-0.29**	-0.37**
Sensory nucleus					
VP	-0.32**	-0.36**	-0.37***	-0.39***	-0.40***
Motor nuclei					
VA	-0.13	-0.20	-0.18	-0.14	-0.18
VL	-0.03	-0.13	-0.11	-0.15	-0.12
Non-specific					
CM/Pf	-0.32**	-0.31**	-0.35**	-0.31**	-0.37**
Fractional Anisotropy					
Association Nuclei					
MDn	0.03	0.01	0.01	0.09	0.03
LP	0.05	0.06	0.05	0.03	0.05
Pu	004	-0.00	0.00	0.06	0.04
Limbic Nuclei					
AP	0.13	0.07	0.08	0.18	0.12
LD	0.31**	0.29**	0.26*	0.24*	0.30**
Sensory Nucleus					
VP	0.16	0.14	0.28*	0.14	0.19
Motor nuclei					
VA	-0.05	-0.03	0.09	0.05	0.01
VL	0.12	0.16	0.11	0.08	0.13
Non-specific nuclei					
CM/Pf	-0.07	-0.06	0.01	-0.09	-0.05
Volume Total – ICV corrected					
Association					
MDn	0.09	0.04	-0.01	0.03	0.04
LP	0.01	-0.00	0.02	-0.07	-0.01
Pu	0.05	0.04	0.09	0.07	0.06
Limbic					
AP	-0.15	-0.20	-0.14	-0.24*	-0.21
LD	0.08	0.04	0.13	0.10	0.08
Sensory					
VP	0.01	0.04	0.02	0.02	0.02
Motor					
VA	0.16	0.16	0.12	0.11	0.15
VL	-0.10	-0.08	0.04	-0.13	-0.07
Non-specific					
CM/Pf	0.10	0.15	0.15	0.10	0.14

Pearson correlations (*r*) between thalamic nuclei measures and cognition in PD patients. Significant correlations are indicated as follows: *$p < 0.05$; **$p < 0.01$; *** $p < 0.001$.

8.7.5.2 Regression modelling results

Mean diffusivity measures of the thalamic nuclei were considered to be the most sensitive to cognitive changes within the PD groups and were further examined using multiple regression analyses. Individual nuclei were highly intercorrelated on this measure (*Table 8-7*) so a series of stepwise linear regressions were applied to the nuclei data with cognitive domain as the dependent variable to establish the independent association between each thalamic nucleus and each cognitive domain.

Table 8-7: The association between thalamic nuclei (MD measure)

	Association		Limbic		Sensory	Motor		Non-specific
	LP	Pu	AP	LD	VP	VA	VL	CM/Pf
Association								
MDn	0.41***	0.31**	0.46***	0.18	0.51	0.45***	0.38***	0.43***
LP		0.19	0.39***	0.11	0.42***	0.36***	0.21	0.31
Pu			0.17	0.09	0.40***	0.31**	0.21	0.17
Limbic								
AP				0.23*	0.43***	0.37***	0.24*	0.19
LD					0.02	0.05	0.03	0.07
Sensory								
VP						0.58***	0.60***	0.44
Motor								
VA							0.77***	0.19
VL								0.31

Pearson correlations (*r*) between thalamic nuclei in PD patients. Significant correlations are indicated: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

For each cognitive domain two models were created. In the first backward stepwise regression the nuclei that had an association with that cognitive domain (*Table 8-6*) were entered as independent variables. In the second model the covariates: age, education, depression and disease duration were added. The proportion of variance (R^2) in cognitive domain was calculated for Model 1 to determine the cumulative effect of nuclei and for Model 2 to determine if the relationship between the thalamic nuclei and cognitive domain survived the inclusion of covariates. Each model also provided the unique proportion of variance each nucleus contributed to the cognitive domain score. The motor nuclei (VA and VL) were excluded from these analyses as they did not have an association with any of the cognitive domains. Results are presented in *Table 8-8.* In the first MD model attention score was the dependent variable and all nuclei except the AP and LP and the motor nuclei (MDn, CM/Pf, LD, VP and Pu) were entered. This model accounted for a significant proportion of the variance in domain scores and the LD, CM/Pf and Pu were all an independent influence on the attention scores (Beta values are presented in parentheses).

This same strategy was applied to the executive function, learning and memory, visuospatial/visuoperception and to the aggregate global score where all the thalamic nuclei except the motor nuclei (AP, MDn, CM/Pf, LD, VP, LP, Pu) were entered into the model. Each model accounted for a significant proportion of the variance in domain scores and had at least two nuclei which were independent predictors of domain score. The LD and CM/Pf nuclei were the most common predictors of cognition, the LD was involved in all cognitive domains and the CM/Pf in attention and visuospatial domains and with global Z score.

When covariates were added into the second model additional variance was accounted for in each domain, although this increase was only significant for the executive function domain and the overall global Z score. Within the attention, learning and memory and visuospatial domains all nuclei that were previously implicated continued to predict cognitive score. Within the executive function domain the LD was no longer a predictor after the inclusion of covariates and for global Z score the only remaining nucleus was the LD.

Some FA and volume measures of nuclei also had an association with cognitive domain so the same multiple regression analyses were applied in order to determine their individual influence on cognition. For FA values, although the white matter integrity of the LD nucleus had a significant association with all cognitive domains and the aggregate global domain when examined using Pearson correlations, this result was not upheld in the regression models. In regards to the visuospatial/perception domain Model One accounted for 7% of the variance in domain score, [$F(2,105) = 3.9$, $p = 0.02$]. Within this model, only the LD nucleus was a unique predictor, accounting for 5% of the unique variance ($p = 0.02$). When covariates were added in the second model, an additional 20% ($p < 0.001$) of the variance ($R^2 = 30\%$, $p < 0.001$) was accounted for by the covariates but LD was no longer an independent predictor of domain score. For the other cognitive domains, Model One was not computed as LD was the only nucleus that was associated with these domains, making this an unnecessary step. When LD was included alongside the covariates in Model Two LD was no longer a unique predictor for the attention, learning and memory, executive function domains, or for the aggregate global score.

The AP was the only nucleus to show a volumetric association with cognition so the first model was not computed. In the second model where AP was entered alongside the covariates the overall model was not significant and AP was not a unique predictor of attention, [$R^2 = 0.02$, $p = 0.74$].

Table 8-8: Backward stepwise linear regression results for MD measures of nuclei

Entered variables			
Model 1		Model 2	
Nuclei that have a correlation with domain		Nuclei and covariates	
% of variance accounted for by unique predictors			
Attention†			
R^2 = 32%, F(5,78) = 7.31, p<0.01		R^2 = 44%, F(9,74) = 6.45, p<0.01	
CM/Pf (β = ¯0.17)	6 (<0.01)	CM/Pf (β = ¯0.15)	8 (<0.01)
LD (β = ¯0.33***)	15 (<0.01)	LD (β = ¯0.25**)	9 (<0.01)
Pu (β = ¯0.20)	9 (<0.01)	Pu (β = ¯0.18)	5 (0.01)
		Disease duration (β = ¯0.25*)	16
		Depression (β = ¯0.20*)	3
Executive Function			
R^2 = 27%, F(7,76) = 3.94, p<0.01		R^2 = 48%,F(11,72) = 6.1, p<0.01	
LD (β = ¯0.23*)	6 (<0.01)	VP (β = ¯0.18)	4 (p<0.01)
VP (β = ¯0.16)	15 (<0.01)	Age (β = ¯0.14)	3 (0.03)
		Education (β = ¯0.15)	3 (0.04)
		Disease Duration (β = ¯0.34***)	4 (<0.01)
		Depression (β = ¯0.29**)	8 (<0.001)
Learning and Memory			
R^2= 30%, F(7,76) = 4.7, p<0.01		R^2 = 38%, F(11,72) = 3.95,p<0.001	
MDn (β = ¯0.25*)	19 (<0.001)	MDn (β = ¯0.18)	4 (0.03)
LD (β = ¯0.24*)	5 (0.03)	LD (β = ¯0.20*)	8 (<0.01)
VP (β = ¯0.20)	4 (0.03)	VP (β = ¯0.23)	15 (<0.001)
		Depression (β = ¯0.24*)	
Visuospatial/Visuoperception			
R^2 = 35%, F(7,76) = 5.80, p<0.001		R^2 = 42%, F(11,72) = 4.7, p<0.001	
CM/Pf (β = ¯0.16)	4 (0.04)	CM/Pf (β = ¯0.16)	4 (0.03)
LD (β = ¯0.34***)	1 (<0.001)	LD (β = ¯0.32**)	8 (<0.001)
		Disease Duration (β = ¯0.23*)	5 (0.01)
Global Z Score			
R^2 = 38%, F(7,76) = 6.5, p<0.001		R^2 = 51%, F(11,72) = 6.8, p<0.001	
MDn (β = ¯0.18)	19 (<0.001)	LD (β = ¯0.25**)	5 (<0.01)
CM/Pf (β = ¯0.17)	4 (0.04)	Depression (β = ¯0.23**)	7 (<0.01)
LD (β = ¯0.32**)	9 (<0.01)	Disease Duration (β = ¯0.27**)	8 (<0.01)
Pu (β = ¯0.16)	4 (0.03)		

Two linear models were used to gauge the proportion of variance accounted for in each cognitive domain. † All nuclei except the motor nuclei were included in each model except for in the attention domain where neither the AP nor the LP nucleus had a correlation with attention scores so were not included. R^2 = proportion of variance accounted for. Only some nuclei in each domain were independent predictors of cognition. Significant beta values are denoted: *$p < 0.05$; **$p < 0.01$; *** $p < 0.001$.

8.7.6 Using thalamic measures to discriminate between groups

Receiver operating characteristics were conducted using both integrity (FA, MD) and volume measures of all nuclei in order to determine the most sensitive region for the separation of the dementia patients (from the control, unimpaired and mild cognitive impaired patients) and of the mild cognitive impairment patients (from control and unimpaired patients). In order to determine the effects of Parkinson's disease alone RoC

analysis was also conducted between the control subjects and PD patient group with no impairment. Results are presented in *Table 8-9*.

8.7.6.1 Identification of the patient group

The Parkinson's disease group without cognitive impairment had very similar thalamic characteristics to the healthy control group. Although volume measures of the AP had a significant area under the curve, separation of the two groups was only achieved with 58.8% accuracy (specificity = 83.33%). MD of the Pu also had a significant AUC, but this only resulted in accurate identification of the patient group 39.2% of the time (specificity = 83.33%).

8.7.6.2 Identification of dementia

Relative to healthy control subjects

For all nuclei, mean diffusivity measures were the best thalamic variable to predict the dementia patients. All regions had a significant AUC and were accompanied by high sensitivity levels in most cases - the following sensitivity levels are reported at 80% specificity. MD of the CM/Pf nucleus approached near perfect separation, correctly classifying the dementia patients 93% of the time. Two of the association nuclei (MDn, Pu) also had high AUC values, enabling correct classification of dementia in 86.7% and 80% of cases respectively. The sensory (VP: 66.7%) and motor (VA: 53.3%; VL: 60%) nuclei displayed sensitivity for dementia above chance levels but regions of the limbic (AP: 40%; LD: 46.7%) and association (LP: 33.33%) nuclei displayed poor sensitivity for the dementia group.

Relative to patients with no cognitive impairment (PD-N)

Prediction of the PD-D group relative to the PD-N group was examined to determine the degree to which the groups could be separated when motor symptoms were not a confounding factor. In the majority of cases this was best achieved using MD measures of nuclei. Volume of AP was the only exception, showing a higher AUC than either FA or MD. Despite this, the volume of the AP was not sensitive to dementia, only correctly identifying 20% of cases.

Similar to prediction of PD-D relative to controls MD of the CM/Pf region had the highest AUC values when predicting PD-D relative to PD-N in this model. Despite this, correct identification of the dementia group was only achieved 40% of the time. Between

patient groups, the effect of motor confounds that is seen in the comparison with the control subjects are reduced and the association nuclei (MDn: 60%; Pu: 66.7%) are better predictors of dementia than either the VL (60%) or the VA (46.7%) nuclei. No other region could correctly identify dementia much more than half the time (VP; 53.33% LP: 33.3%; LD: 53.3%).

Relative to patients with mild cognitive impairment

Prediction of the PD-D group relative to PD-MCI was not highly accurate. Only three regions showed a significant area under the curve, and all only when measured with mean diffusivity. The CM/Pf, (AUC: 0.81, $p < 0.001$) could only accurately identify PD-D 40% of the time, the VP (AUC: 0.71, $p = 0.02$) 46.7% of the time with the VL (AUC: 0.74, $p = 0.01$) the worst region at 26.7% sensitivity.

Table 8-9: Group separation by integrity measures of nuclei, RoC results

	PD motor	Mild cognitive impairment		Dementia		
	C vs PD-N	C vs PD-MCI	PD-N vs PD-MCI	C vs PD-D	PD-N vs PD-D	PD-MCI vs PD-D
Association nuclei						
MDn						
Vol	0.51ns (0.39–0.62)	0.52ns (0.36–0.68)	0.51ns (0.38–0.63)	0.57ns (0.40–0.73)	0.57ns (0.45–0.70)	0.60ns (0.41–0.76)
FA	0.57ns (0.45-0.68)	0.57ns (0.41–0.72)	0.51ns (0.39–0.64)	0.56ns (0.3–0.72)	0.53ns (0.40–0.67)	0.55ns (0.37–0.73)
MD	0.54ns (0.42–0.65)	0.73[b] (0.58-0.86)	0.69[b] (0.57-0.80)	0.86[c] (0.72-0.95)	0.83[c] (0.71-0.91)	0.68ns (0.50 – 0.83)
Pu						
Vol	0.52ns (0.40–0.64)	0.59ns (0.42–0.74)	0.58ns (0.46 – 0.70)	0.51ns (0.35–0.67)	0.54ns (0.41–0.66)	0.60ns (0.42–0.77)
FA	0.60ns (0.48-0.71)	0.54ns (0.38–0.69)	0.60 ns (0.48-0.72)	0.54ns (0.38–0.70)	0.56ns (0.43–0.68)	0.52ns (0.34–0.69)
MD	0.70[b] (0.58-0.80)	0.75[b] (0.59-0.87)	0.59ns (0.47 – 0.71)	0.80[b] (0.64-0.91)	0.72[a] (0.60-0.82)	0.63ns (0.45 – 0.79)
LP						
Vol	0.53ns (0.42–0.65)	0.60ns (0.44–0.75)	0.64 ns (0.52-0.75)	0.56ns (0.39–0.71)	0.60ns (0.48–0.72)	0.56ns (0.38-0.73)
FA	0.57ns (0.45–0.69)	0.56ns (0.40–0.71)	0.53ns (0.40–0.65)	0.61ns (0.44–0.76)	0.56ns (0.43–0.68)	0.56ns (0.38–0.73)
MD	0.57 ns (0.45-0.69)	0.62 ns (0.46-0.77)	0.62ns (0.49–0.73)	0.71[a] (0.54-0.84)	0.70[b] (0.58-0.81)	0.60ns (0.42 – 0.77)
Limbic nuclei						
AP						
Vol	0.71[c] (0.59-0.81)	0.59ns (0.43-0.74)	0.68[c] (0.55-0.79)	0.53ns (0.37-0.69)	0.68[a] (0.55-0.79)	0.57ns (0.39-0.74)
FA	0.52ns (0.40-0.63)	0.60ns (0.44-0.75)	0.57ns (0.44-0.69)	0.58ns (0.41-0.74)	0.57ns (0.44-0.69)	0.54ns (0.36-0.72)
MD	0.59ns (0.47–0.70)	0.61ns (0.45-0.76)	0.62ns (0.49-0.73)	0.68[a] (0.51-0.82)	0.62ns (0.49-0.73)	0.57ns (0.38-0.74)
LD						
Vol	0.53ns (0.41-0.64)	0.51ns (0.35-0.67)	0.51ns (0.38-0.63)	0.53ns (0.36-0.69)	0.51ns (0.38-0.63)	0.54ns (0.36-0.71)
FA	0.55 ns (0.43-0.66)	0.73[b] (0.57-0.86)	0.69[c] (0.57-0.80)	0.70[a] (0.53-0.84)	0.69[b] (0.57-0.80)	0.52ns (0.34-0.69)
MD	0.51ns (0.39-0.62)	0.73[b] (0.58-0.86)	0.72[b] (0.60-0.82)	0.79[c](0.63-0.90)	0.80[c] (0.68-0.89)	0.52ns (0.34-0.70)
Sensory nucleus						
VP						
Vol	0.54ns (0.42-0.66)	0.60ns (0.44-0.75)	0.56ns (0.43-0.68)	0.52ns (0.35-0.68)	0.54ns (0.41-0.66)	0.59ns (0.41-0.76)
FA	0.63 ns (0.51-0.74)	0.56ns (0.39-0.71)	0.55ns (0.43-0.67)	0.51ns (0.35-0.68)	0.60ns (0.47-0.72)	0.53ns (0.35-0.71)
MD	0.55ns (0.43-0.67)	0.62 ns (0.46-0.77)	0.58 ns (0.46-0.70)	0.84[c] (0.69-0.94)	0.80[c] (0.68-0.88)	0.71[a] (0.52-0.85)
Non-specific nucleus						
CM/Pf						
Vol	0.53ns (0.41-0.65)	0.54ns (0.37-0.69)	0.51ns (0.38-0.63)	0.59ns (0.42-0.74)	0.62ns (0.49-0.74)	0.64ns (0.46-0.80)
FA	0.52ns (0.40-0.63)	0.53ns (0.37-0.69)	0.52 ns (0.40-0.65)	0.53ns (0.36-0.69)	0.60ns (0.47-0.72)	0.51ns (0.33-0.69)
MD	0.61 ns (0.49-0.72	0.62 ns (0.46-0.76)	0.51ns (0.39-0.63)	0.92[c] (0.79-0.98)	0.83[c] (0.72-0.91)	0.81[c] (0.64-0.93)

	PD motor	Mild cognitive impairment		Dementia		
	C vs PD-N	C vs PD-MCI	PD-N vs PD-MCI	C vs PD-D	PD-N vs PD-D	PD-MCI vs PD-D
Motor nuclei						
VA						
Vol	0.50ns (0.39-0.62)	0.62ns (0.46-0.77)	0.62ns (0.49-0.73)	0.63ns (0.46-0.78)	0.62ns (0.49-0.74)	0.54ns (0.36-0.72)
FA	0.62 ns (0.50-0.73)	0.55ns (0.39-0.70)	0.56ns (0.43-0.68)	0.59ns (0.42-0.74)	0.56ns (0.44-0.69)	0.52ns (0.34-0.70)
MD	0.58ns (0.47-0.70)	0.74[b] (0.58-0.86)	0.66[a] (0.54-0.77)	0.74[b] (0.57-0.87)	0.68[a] (0.55-0.79)	0.52ns (0.34-0.69)
VL						
Vol	0.55ns (0.43-0.67)	0.58ns (0.42-0.73)	0.54ns (0.42-0.66)	0.59ns (0.42-0.74)	0.55ns (0.42-0.67)	0.50ns (0.33-0.68)
FA	0.54ns (0.42-0.66)	0.57ns (0.40-0.72)	0.60 ns (0.47-0.72)	0.58ns (0.41-0.73)	0.65[a] (0.52-0.76)	0.52ns (0.34-0.70)
MD	0.59 ns (0.47-0.70)	0.63ns (0.47-0.78)	0.56ns (0.43-0.68)	0.87[c] (0.72-0.95)	0.79[c] (0.68-0.88)	0.74[b] (0.56-0.88)

AUC: area under the receiver operating curve (chance = 0.5; perfect separation = 1.0). Superscript numbers indicate level at which the comparison is significant: a $p < 0.05$; b $p < 0.01$; c $p < 0.001$.

8.7.6.3 Identification of PD mild cognitive impairment

Relative to healthy control subjects

MD remained the best variable to predict cognitive impairment. The lower level of motor impairment in the PD-MCI group resulted in even the motor nuclei being unable to separate the two groups (VA: 50%; VL: 33.3%). The cognitive nuclei were also of limited value, with only the association nuclei (MDn, Pu) able to correctly identify cognitive decline more often than chance (55.6% for both regions). For every other nucleus AUC levels were either not significant (AP, LP, CM/Pf, VP) or sensitivity level was only 50% (LD).

Relative to patients with no cognitive impairment

Arguably the most important comparison, that of the unimpaired patients and those with mild cognitive impairment was best achieved using diffusivity measures in the majority of cases. The only exception which reached significance was again in the AP nucleus where volume had a higher AUC (33.3%) than MD or FA. For the first time, the LD nucleus was the best discriminatory ROI here, although this only resulted in correct identification of PD-MCI in 55.6% of cases. None of the other regions associated with cognition had a significant AUC (CM/Pf, VP, LP, Pu), or could not predict PD-MCI at higher than chance levels (MDn). Not surprisingly, the motor nuclei (VA: 44.4%; VL: 38.9%) could also not discriminate between groups at higher than chance levels.

8.8 Discussion

8.8.1 Summary of the results

The preceding chapter addressed the idea that the regions of the thalamus are not affected in unison and instead exhibit differential involvement in the symptoms of PD. In line with the results of a prior study into thalamic integrity in PD (Peran, et al., 2010), we have shown that examining the structural integrity of the thalamic nuclei using diffusion parameters provides a better reflection of thalamic disruption than measures of gross structural atrophy in PD does. In addition, we have confirmed the relationship between all thalamic nuclei and multiple domains of cognition in a single sample for the first time. Our results support two previous studies that have reported a relationship between cognition and some thalamic regions in a Schizophrenia (Hazlett, et al., 1999) and Alzheimer's (Qiu, Fennema-Notestine, et al., 2009) sample and one where the anterior and medio-dorsal nuclei were independently segmented from the rest of the thalamus shown to have a strong relationship with related cognitive domains (Gilbert, et al., 2001). Finally, we have shown that the thalamic nuclei are differentially targeted and begin showing degeneration according to the vulnerability of the cortical regions they have primary connectivity with. The limbic nuclei, reciprocally connected with the frontal lobes (Jones, 2007a) are the most affected and show the signs of subtle degeneration at the cellular level in PD patients with no detectable cognitive impairment before exhibiting gross levels of atrophy in those with dementia. These results confirm what our group (Melzer, et al., 2011a), and others (Beyer, Janvin, et al., 2007; Burton, et al., 2005) have found in regards to frontal lobe disruption occurring in the early stages of PD. We have shown that the association nuclei and the centromedian/parafascicular complex, previously heavily implicated in PD (Henderson, et al., 2000a, 2000b) and PD-D (Brooks & Halliday, 2009) at autopsy show disruption in those patients with only mild cognitive impairment and have a strong association with multiple cognitive domains in this sample. The sensory and motor nuclei are only involved in those patients with dementia, in line with histology studies that show minimal pathology in these regions in PD (Braak, et al., 2003).

Mean diffusivity, fractional anisotropy and volume of the thalamic nuclei were examined to give a detailed impression of nuclei degeneration in PD. In most instances the mean diffusivity measures of thalamic nuclei were more reflective of the cognitive state of the PD patients, showed a greater association with domains of cognition and allowed for

markedly better discrimination between cognitive groups than either volume or fractional anisotropy measures. These results confirm those reported in *Chapter 7* where diffusivity measures of the whole thalamus showed significant change between cognitive groups with only mild cognitive dysfunction and dementia, prior to evidence of gross atrophy in the PD-D group. The only exception was in the LD nucleus where FA was a better predictor of cognition. In all other nuclei except the motor nuclei, increased diffusivity was observed in the PD-D group, and in the case of the MDn and LD nuclei, also in the PD-MCI group relative to PD-N patients in the absence of volume loss.

Increased MD is the result of disruption in neurons, axons or supporting glial cell structures (Assaf & Pasternak, 2008) within the thalamic nuclei and is most likely caused by neuronal death or LB pathology (Brooks & Halliday 2009). LB pathology especially plays an important part in the development of dementia in PD (Aarsland, et al., 2005; Hurtig, et al., 2000) and is thought to be one of the main contributions to the MD results within this study.

In the thalamic nuclei that are associated with cognitive function mean diffusivity measures of degeneration are increased in those areas known to be infiltrated with LB pathology. The association (MDn, LP and Pu) nuclei show medium level infiltration (Braak & Braak, 2000; Rub, Del Tredici, Schultz, et al., 2002), with the LP the worst affected. The CM/Pf complex is undoubtedly the most affected by pathology and has heavy infiltration levels in the CM and only slightly less in the Pf. This appears to contribute to cellular degeneration as both regions also exhibit a great degree of neuronal loss and the Pf, gross volume loss (Henderson, et al., 2000a). It is interesting to note then, that in our study these regions also show the greatest change in mean diffusivity between cognitive groups. The MDn nucleus is the only region to show a significant progressive increase in diffusivity from PD-N to PD-MCI to PD-D and, along with the VP – the CM/Pf one of the only regions to show a MD increase from PD-MCI to PD-D. As histology has not been carried out on the VP nucleus of PD patients to date, we can only assume that LB pathology is also present in this region. MD levels in the pulvinar also show an association with cognition in our sample, there is a significant increase in MD in both the PD-D and PD-MCI group relative to control subjects, as well as in the PD-D group relative to PD-N. In the LP, a similar result is seen with a significant MD increase in PD-D relative to controls and PD-N.

The VA and VL motor nuclei reportedly show light LB pathology (Braak & Braak, 2000), and in this sample also show a significant increase in diffusivity between the PD-D

group and control subjects. No changes are observed in comparison between any other PD groups, indicative of the fact the VA and VL have no association with cognition. The AP nucleus reportedly shows no LB pathology, neuronal or volume loss (Braak & Braak, 2000), and not surprisingly also shows no bilateral MD changes within the PD cognitive groups in this sample. In the right hemisphere however the AP has significant cellular disruption in PD-N patients, perhaps suggesting differential LB infiltration between hemispheres although this is a difficult issue to explore as to date, no histological studies have provided a comparison between hemispheres of neuronal count. Peran, (2009) has previously reported a significant effect of lateralisation in the thalamus however, where the right thalamic volume was larger than the left in an ageing cohort and this could indicate degeneration occurs in one hemisphere prior to the other.

8.8.2 The relationship between thalamic nuclei and cognition

Using robust statistical analyses we confirmed the association between thalamic nuclei and specific areas of cognition in some cases, but did not find an association where expected in others. We did successfully extrapolate cognitive influence from the contaminant of motor dysfunction in PD better than has been attempted previously however. All nuclei showed a strong association with at least one area of cognition, with the combined effect of several nuclei showing a markedly increased contribution to specific areas of cognitive function.

Although volume of the AP nucleus and FA of the LD and VP nuclei had a correlation with some areas of cognition, mean diffusivity was the best independent predictor of cognitive function in all cases. When covariates were controlled for using multiple regression analysis the relationship between volume and FA measures of nuclei did not survive. Mean diffusivity measures of the individual thalamic nuclei on the other hand showed an independent contribution to specific domains of cognition while the combined effect of groups of thalamic nuclei further increased contribution in most cases. One recent DTI study has reported decreased FA of the MDn nucleus as a neurocorrelate of depression (Li, et al., 2010), but diffusivity measures of thalamic nuclei have not previously been examined in relation to cognition in PD.

The notion that cellular disruption in thalamic nuclei causes cognitive dysfunction is somewhat supported by the above autopsy examinations of LB pathology (Braak, et al., 2000; Henderson, et al., 2000a; Rub, Del Tredici, Del Turco, et al., 2002). In all three samples any patients that were exhibiting dementia prior to death were excluded from the investigation. This could perhaps explain the lack of abundant pathology in wider thalamic

regions. Prior to the development of dementia, it is now well established that most PD patients will exhibit lower levels of cognitive dysfunction, even in the early stages (Aarsland, et al., 2009). There is no guarantee that patients in any of the above samples were not exhibiting some degree of cognitive impairment as none of the studies report conducting extensive neuropsychological tests. This hypothesis is somewhat supported by the fact the Henderson, et al., (2000a) sample reports an average disease duration of 7.2 years and the Rub, et al., (2002) sample an average Hoehn and Yahr stage of 4-5. MCI has been previously diagnosed in a PD sample with average Hoehn and Yahr stage of 2.6 (Beyer, Janvin, et al., 2007) with reduced mini mental status is evident in a sample of PD patients from the same group who had a disease duration of 6 years (Beyer & Aarsland, 2008). Data from our own cohort also supports the possibility of MCI at this length of disease duration and Hoehn and Yahr staging. In one of our cohorts, MCI was diagnosed in a group with average disease duration of 7.3 years and a Hoehn and Yahr stage of 2.6 (Dalrymple-Alford, et al., 2010). The degree of LB pathology in these samples therefore is a likely correlate of early cognitive dysfunction, and can be reflected in the cellular disruption that is shown by mean diffusivity levels.

MD parameters of thalamic nuclei show an association with multiple domains of cognition, although this is primarily domain-specific with each region influencing the cognitive subtype that is moderated by those areas of the cortex that they show the greatest reciprocal connectivity with. This domain-specific association confirms a recent study (Sasson, Doniger, Pasternak, Tarrasch, & Assaf) which used combined DTI and VBM analysis of the whole brain to examine the relationship between cognition and specific cortical regions in healthy control subjects. Participants were healthy, aged 25-82. Cognition, separated into domains of executive function, information processing speed and memory was examined using a computerised neuropsychological test battery. Results are similar to our own, where our nuclei that have frontal connectivity correlate with executive function and attention, the Sasson et al., (2012) study showed partial correlations between executive function score and diffusion parameters in the left inferior frontal gyrus. Similarly, in our nuclei that have temporal connectivity we demonstrated a correlation with memory, and Sasson, et al., a relationship between memory and temporal and frontal regions including the right inferior frontal gyrus, medial frontal gyrus and superior longitudinal fasciculus – the white matter tract which links the parietal and temporal lobes to the frontal lobes. This study lends significant support to the idea that the microstructural integrity of the cortex is heavily associated with cognition.

8.8.2.1 Attention

The limbic AP (Aggleton & Brown, 1999) and LD (Edelstyn, et al., 2006) thalamic nuclei are considered to be primarily influential over the learning and memory domains of cognition. It was also expected that the AP would play a significant role in the cognitive domain of attention due to its largely prefrontal afferent connectivity (Aggleton & Brown, 1999). The role of the anterior nuclei complex in attention is supported by the fact that this complex shows increased activation in patients suffering from prepulse inhibition – a neurological phenomenon which manifests in the inability to filter out unnecessary information, or, pay attention to target information (Hazlett, et al., 2001).

Our results show that the LD was involved in the attention domain but the AP was not. Attention was mainly predicted by the LD, but it was the combined influence of the AP, CM/Pf and Pu nuclei that accounted for the greatest proportion of variance in attention domain score.

The CM/Pf complex accounted for only slightly less of the variance in attention domain scores than the LD nucleus. Although the CM/Pf has traditionally been considered non-specific due to the diffusivity of its connectivity (Jones, 2007b), this result confirms those of Kinomura, et al., (1996) who showed that in healthy control subjects the CM/Pf complex was the only thalamic region to show increased blood flow during the performance of an attention task. Our results also implicate the Pu on attention tasks. These results were not unexpected as the Pu is primarily involved in visuospatial function. As all attention tasks in our neuropsychological test battery (*Section 5.3*) required visual interpretation we can assume Pu disruption would manifest as a deficit on these tests, a result that is in line with previous research showing Pu involvement when a participant is required to only attend to a stimulus and not perform any other tasks (Kastner, et al., 2004; LaBerge & Buchsbaum, 1990).

8.8.2.2 Executive function

We expected a significant association between the MDn nucleus and the executive function domain, a result that was not upheld here. Surprisingly, the VP nucleus was the only independent predictor of this domain, although the LD nucleus was also involved before the influence of covariates was considered. The connectivity between the VP and the secondary somatosensory cortex may account for this result. Terminations of the VP include regions of the secondary somatosensory cortex (Bhatnagar, 2002) and this region

has previously shown activation during tasks of executive function in healthy control participants (Kircher, Nagels, Kirner-Veselinovic, & Krach, 2011).

The neuropsychological tests applied here to measure executive function included measures of fluency - specifically category, letter and action fluency (*See Section 5.3.4.2*) where participants were given a letter or category and asked to name as many objects beginning with that letter or within the category as quickly as possible. The relationship between executive function and the VP was primarily driven by the letter fluency task where participants were required to name as many words as they could think of beginning with a certain letter. Follow up analysis of each test independently indicated that this task was the only neuropsychological test to show a relationship ($r = 0.25$, $p = 0.02$) with the VP nucleus, and that the VP also remained a significant independent predictor of the letter fluency task after the inclusion of covariates ($r^2 = 6\%$, $p = 0.02$). In the healthy control study (Kircher, et al., 2011), although the cerebellum, frontal and temporal lobes were all activated during the performance of any verbal fluency task, letter fluency was shown to be the specific domain of the parietal lobe. We have therefore concluded that the association between VP and letter fluency in this sample is due to the connectivity between the VP and several areas within the secondary somatosensory cortex, specifically areas within the parietal lobe.

8.8.2.3 Learning and Memory

As expected, the MDn and LD influenced the learning and memory domain, although somewhat counterintuitively, the VP nucleus was the biggest contributing factor here. The influence of the MDn and LD over learning and memory confirms results from several previous studies. In regards to the MDn, this region has been consistently implicated in Schizophrenia (Qiu, Zhong, et al., 2009) and in healthy control subjects (Johansen-Berg, et al., 2005), showing a strong relationship with executive function and working memory tasks in both studies. Similarly, the LD has been previously implicated in recall memory (Edelstyn, et al., 2006) in a single subject with LD lesions. In addition, the LD nucleus has reciprocal connectivity with the limbic cortex and the cortices surrounding the hippocampus and is part of the extended hippocampal pathway which mediates learning and memory processes (Edelstyn, et al., 2006) confirming that degeneration in this area will correspond to deficits in memory processes.

In regards to the VP, due to axons primarily terminating in the somotosensory cortices, (Bhatnagar, 2002) we expected this region to show an association with sensory

deficits. The somatosensory cortex has previously been implicated in a PD sample (Brefel-Courbon, et al., 2005), where higher somatosensory cortex activation and lower pain threshold was evident in PD patients compared to controls during the administration of painful stimuli. Surprisingly, we could not confirm this association. Although there was a significant association between VP and UPDRS III score ($r = 0.30$, $p = 0.01$) in our cohort, there was no association between the sensory subset of UPDRS ($r = -0.07$, $p = 0.54$) and the VP.

Follow up analysis was again employed to examine the specific subsets of learning and memory to examine which specific test accounted for the strong influence of the VP within this domain. Individually, none of the cognitive tests had an association with the VP despite the overall significant relationship between the VP and the aggregate learning and memory score. This lack of association suggests that the VP influence over learning and memory is greater than the sum of the influence of individual tasks. Another factor is likley mitigating the effect of the VP in this cognitive domain. This could be other thalamic nuclei that have connectivity with the VP, or it might be that the connectivity between the VP and other regions is disrupted, preventing adequate performance of these tasks.

Our own fibre tracking results which will be explored in depth in the subsequent chapter, indicate that connectivity of the VP does primarily terminate in the somatosensory cortices. Prior to reaching this destination however, fibres appear to be concentrated in regions of the medial temporal lobe, with dense projections cumulating in the hippocampal region. As the main function of the hippocampus is memory consolidation (Alvarez & Squire, 1994) it is possible that disruption within these axonal tracks has affected the normal function of the hippocampus in this sample, which has lead to the observed deficits in learning and memory and the contribution of the VP nucleus to this domain.

8.8.2.4 Visuospatial/Visuoperception

Interestingly neither the LP nor Pu were involved in visuospatial or perception ability as expected, with the CM/Pf appearing to be primarily responsible for this. This result is in contrast to the findings of (Ricker & Millis, 1996) who showed significant LP and Pu involvement in visuospatial/perception in patients with posterior thalamic lesions. As this group only examined these posterior nuclei and did not contrast results with changes in any other regions of the thalamus it is difficult to conclude that visuospatial function is solely the domain of the LP and Pu however.

The combined involvement of the CM/Pf and LD suggests that frontal disruption is the primary cause of visuospatial and perception dysfunction in PD. The CM/Pf complex and the LD nuclei were the only regions to show an independent association with this cognitive domain. The primary projections of the CM/Pf are to the striatum but diffuse connectivity is also evident throughout the cortex, and in PD, degeneration of a small nucleus surrounding this region is associated with dementia (Brooks & Halliday 2009). Visuoperception is impaired in in some PD-D patients (Janvin, et al., 2003) and is a likely mitigating factor underlying other cognitive domains as visual interpretation is required for nearly all neuropsychological tests.

The LD nucleus has similar prefrontal and frontal connectivity as the AP and can also be considered as part of the limbic system extended hippocampal pathway (Edelstyn, et al., 2006). As a deficit in visual perception corresponds to activation of the medial temporal lobe (Holt, et al., 2006) the disruption in the LD could be hypothesised to cause the visual spatial/perception deficits observed here due to a breakdown in communication with the temporal lobe.

The visuospatial/perception domain encompassed examination of spatial awareness and stimuli perception, functions that were able to be independently examined in most cases. In regards to the visuospatial deficits, the judgement of line orientation test (JOL) primarily examined spatial awareness. LD was not an independent predictor of the JOL test after inclusion of covariates. CM/Pf on the other hand, remained a unique predictor of JOL, accounting for 10% ($p < 0.001$) of the variance in the test score. Our results are supported by work with primates, where the CM/Pf is involved in the interpretation of the spatial location of visual stimuli (Minamimoto & Kimura, 2002). In monkeys, the reaction time required to detect a target was decreased if the presentation of the target was preceded by a warning stimulus in the same spatial location, compared to in an opposite location of the target. During presentation of the warning stimulus the neurons in the CM/Pf complex showed strong activation. Lesioning of the CM/Pf complex rendered the monkeys completely unable to interpret the visual stimuli. The generalisation of this result to human subjects is somewhat limited by results from a single subject who had experienced a left thalamic haemorrhage which caused damage to the CM/Pf and VL nuclei. Although the patient experienced significant motor dysfunction, no spatial neglect was evident when he was asked to copy a three dimensional structure or draw a picture of a clock or a room (Manabe, Kashihara, Ota, Shohmori, & Abe, 1999).

Visual perception was examined using the visual objects and space perception (VOSP) fragmented letters test. Both the LD (r^2 = 4%, p = 0.04) and CM/Pf (r^2 = 5%, p = 0.03) nuclei were influential over this domain, with a significant proportion of the variance in test scores accounted for by these regions after the inclusion of covariates in the multiple regression. Similarly, both the LD (r^2 = 15%, $p < 0.001$) and CM/Pf (r^2 = 7%, p = 0.01) contributed to the copy subset of the Rey Complex Figure Test (RCFT), a test encompassing both spatial and visual perception. The fact that both nuclei were influential in these domains suggests that the influence over visual perception is mediated by frontal connectivity as both the LD and CM/Pf have extensive frontal connectivity, but do not generally share connectivity with any other cortical regions.

8.8.2.5 Global Z score

Finally, in regards to the aggregate measure of global functioning, the LD nucleus was the only region that had an independent association with this score. Prior to inclusion of the covariates however, the association (MDn, Pu) and CM/Pf nuclei were also heavily involved. This suggests the aggregate score is largely reflective of frontal dysfunction as all these regions, except the Pu (Highley, et al., 2003) have demonstrated primarily frontal connectivity (Jones, 2007a). In this case, either the CM/Pf or LD, sometimes in conjunction, primarily influenced the level of global cognitive, rather than specific domains of dysfunction in this cohort. If the mini mental status exam can be considered to be representative of global function, the idea of frontal disruption influencing global cognitive status has been confirmed from previous studies. The Burton et al., (2004) cohort showed that grey matter atrophy was restricted to the frontal lobes in PD patients relative to controls. The PD patient group excluded those with dementia but, on average the group demonstrated a lower MMSE score (26.4) compared to the control group (28.1) so this could indicate that frontal dysfunction in this instance is largely reflective of early cognitive decline. Similarly, a later PD cohort that was examined by the same group (Beyer, Larsen, et al., 2007) showed largely frontal, but also some temporal trend level grey matter loss in PD-MCI relative to healthy control subjects. Using more extensive neuropsychological testing, our own group (Melzer, et al., 2011b) has also demonstrated that frontal lobe disruption is the biggest correlate of global function, showing grey matter loss in largely frontal, but also some temporal regions in PD-MCI compared to controls, worsening in PD-D. An association between aggregate global Z score and extensive regions of the frontal and few regions of the temporal cortex was observed in our sample

and the association between the aggregate global measure and MMSE and MoCA was supported, with the patient group exhibiting progressively lower MMSE and MoCA scores from PD-MCI to PD-D.

8.8.3 Nuclei involvement at different levels of cognitive impairment in PD

After examination of all nuclei it is clear that each become involved in the cognitive or motor symptoms of Parkinson's disease at different stages and the limbic nuclei are especially vulnerable. The AP nucleus is disrupted in the very early stages of PD, indicated by the fact celluar disruption is evident in this region in patients without cognitive impairment. This is not to say that each influences a specific behaviour with no interconnection however. Rather, what appears to be happening is the differential pathological infiltration of the thalamic nuclei primarily affects those cortical regions with preferential connectivity, with the influence of degeneration in surrounding, and connected areas involved to a lesser degree. Thus, we should see nuclei degeneration in accordance with LB pathology levels at each stage of PD.

This study measured the level of nuclei degeneration at each cross-sectional stage of PD, with changes in volume, diffusivity or anisotropy assumed to represent the accumulation in disruption that occurs after the previous stage. In the early stages of PD not all nuclei are affected, but most show heavy degeneration in those patients that have dementia in the final stages. The following section of this chapter addresses the changes in thalamic nuclei at each cognitive stage of PD and compares results with evidence gained from other studies.

8.8.3.1 In PD without cognitive impairment

The limbic nuclei are the first to show degeneration in PD. Increased diffusivity is apparent in the AP (right side only) of PD-N subjects relative to controls, with further increases occurring as cognition worsens in PD-MCI and PD-D. AP degeneration is also hypothesised to occur in the early stages of AD, and along with the MDn, is one of the thalamic regions showing the greatest level of cellular disruption in AD (Chen, et al., 2007). Given the level of degeneration, the authors of the Chen et al., (2007) study hypothesised that disruption occurs in the AP earlier and to a greater degree than it does in other thalamic regions.

Degeneration in the AP nucleus was assumed to cause wider communication disruption and be representative of further degeneration in the limbic loop (Braak & Braak,

2000) but despite the early involvement of the AP nucleus in cognitive dysfunction, examination of this region did not aid in discrimination of PD cognitive groups. Although the anterior principal nucleus has not been investigated in PD before, the connectivity of its axons has been investigated by our own group using DTI methods (Melzer, et al., 2011a). In our PD-N group, reduced MD was evident in several fibre tracts, including in the anterior thalamic radiation – the white matter tract which connects the anterior regions of the thalamus to the prefrontal cortex. This suggests that the subtle degeneration observed in the AP in this study is a consequence of, or a contributing factor to the reduced integrity of the white matter tract into the frontal lobes.

Support for this hypothesis comes from a single sample of PD patients. When grey and white matter density, FA and MD were examined in a PD cohort (Kendi, et al., 2008) and compared to healthy controls no decrease in grey or white matter volumes were observed in the patient group. Conversely, a significant decrease in FA was reported for extensive regions of the frontal lobes in patients, in the absence of wider cortical changes. As there was no corresponding change in MD this suggests the axonal integrity is primarily disrupted here, and that other cellular integrity largley remained intact (Watts, 2008). Although the patients in the Kendi, et al., sample did not have dementia, their level of neuropsychological functioning was not examined any further than what was required to exclude dementia. This leads to difficulties in interpretation of their level of cognitive dysfunction. It can be assumed they were not showing significant levels of impairment however as their Hoehn and Yahr score was only 1.8 and disease duration only 5.8 years, clinical characteristics that are very similar to those seen in our own (Dalrymple-Alford, et al., 2010) PD-N sample [H+Y = 1.9 (0.7), DD = 4.6 (3.9)].

8.8.3.2 In PD with mild cognitive impairment

The LD and MDn thalamic nuclei become disrupted in patients with PD-MCI. In the LD, reduced fractional anisotropy and increased mean diffusivity was evident in the PD-MCI group relative to controls but neither variable aided in distinguishing PD-MCI from PD-N. Degeneration of the MDn was slight, only detected by changes in mean diffusivity. The MDn showed an increase in diffusivity relative to both the control and PD-N groups but there was significant overlap between the PD-MCI and PD-N groups. PD-MCI could successfully be identified relative to the control subject's using this variable however.

Little is known about the neuroanatomical substrates of MCI in PD and although we (Melzer, et al., 2011a) have previously implicated the AP in PD-MCI no other thalamic

nuclei have been specifically examined in PD or AD MCI. With their combined prefrontal connectivity however, the LD and MDn disruption can be assumed to represent further frontal lobe degeneration, an idea confirmed by several groups that have used various imaging modalities to investigate MCI in PD. In the Beyer, Janvin, et al., (2007) sample trend level frontal lobe grey matter loss is evident in PD-MCI relative to patients with intact cognition. In our own (Melzer, et al., 2011b) cohort, changes in PD-MCI were examined relative to healthy controls, showing a significant reduction in grey matter throughout the frontal lobes. Similar to the Beyer, et al., (2007) sample, when changes were examined relative to PD-N, grey matter reduction levels did not remain significant. In contrast, a small region of the middle frontal gyrus shows degeneration in the (Song, et al., 2011) PD-MCI group relative to PD with intact cognition.

Frontal atrophy is not a contaminant of Parkinson's disease MCI alone, as it has also been identified in those with neurodegenerative (n-MCI), vascular (v-MCI) and Parkinson's Lewy body MCI (plb-MCI) relative to control subjects (Meyer, Huang, & Chowdhury, 2007). One very interesting study (Lee, et al. 2010b) has also compared single domain amnesic MCI patients (aMCI: diagnosed according to Petersen, et al., (1997) criteria) with and without PD. Frontal lobe degeneration is again heavily implicated in cognitive dysfunction here, as relative to control subjects grey matter loss is evident in the middle frontal and precentral gyrus in PD-MCI. The difference in pre AD and pre PD-D groups in regards to memory impairment was highlighted, as the pre AD aMCI group had more severe impairment on the neuropsychological tests of memory than the pre PD-D aMCI group had. This was reflected in the difference in cortical changes, as the aMCI group without PD showed significant temporal degeneration while these regions were largely spared in the aMCI group with PD.

The MDn also has some connectivity with temporal, parietal and occipital regions of the cortex (Buchsbaum, Buchsbaum, et al., 2006). A comparison with a-MCI with and without PD reports significant perfusion decreases in temporal, parietal and occipital lobes in those with PD compared to those without, and a healthy control group (Nobili, et al., 2009). The temporal lobes especially are implicated in MCI suggesting MDn degeneration is also reflective of degeneration in the wider temporal cortex. In the above samples superior and inferior temporal lobes (Beyer, Larsen, et al., 2007), Heschl's gyri, planum temporale and right insula along with the superior and inferior temporal lobes (Melzer, et al., 2011b), the bilateral temporal horn of those with neurodegenerative MCI and in the wider temporal cortex in those with n-MCI, v-MCI and plb-MCI all exhibited significant

grey matter atrophy relative to control subjects (Meyer, et al., 2007). Temporal degeneration is reflective of multiple domain mild cognitive impairment in PD. In a group with multiple domain mild cognitive impairment, cortical atrophy was more extensive and included further regions in the temporal as well as the frontal lobes than were implicated in single domain cognitive impairment (Lee, et al., 2010a).

As mentioned previously, the anterior thalamic radiation shows significant disruption in PD-N relative to controls. Not surprisingly, the integrity of this tract further worsens in PD-MCI (Melzer, et al., 2011a). In one of the few other studies to apply diffusion tensor imaging to PD-MCI (Hattori, et al., 2011) several other white matter tracts were also found to be disrupted in PD-MCI relative to controls, including the inferior fronto-occipital fasciculus which is a white matter tract connecting the frontal lobe to the occipital and temporal lobes (Wanaka, Jiang, Nagae-Poetscher, van Zijl, & Mori, 2004). Interestingly, in this PD sample no grey matter atrophy was identified in PD-MCI in any regions, and the changes in diffusivity of the white matter tract did not remain when examined relative to PD-N, suggesting that degeneration is subtle and occurs as a pre-cursor to grey matter reduction.

The notion of disruption localised to the frontal and temporal lobes in the early stages of cognitive dysfunction is not a new one. Before the concept of PD-MCI was well established Braak, et al., (2003) demonstrated that LB and LN pathology exists in the temporal mesocortex as early as stage 3, worsening in stage 4 and spreading to prefrontal and other regions of the neocortex in stage 5. Later, this group (Braak, Rub, et al., 2006) showed that cognition (MMSE) is impaired as early as stage 3 in some patients, with a significantly greater number of patients showing mildly impaired (MMSE 21-24), moderately impaired (MMSE 11-20) and severely impaired (MMSE 0-10) cognition from stages 4-6.

8.8.3.3 In PD with dementia

The CM/Pf, Pu, LP and VP nuclei all only begin showing significant levels of degeneration once cognitive dysfunction is advanced in PD-D, and were all best measured by MD, indicating disruption is not yet severe enough to be reflected in gross atrophy of these regions. The bilateral diffusivity changes in the CM/Pf is in line with the histology studies conducted by Brooks et al., (2009) who showed degeneration of regions within the intralaminar nuclei exhibit markedly reduced volume in PD-D relative to PD and a strong relationship with cognition in the CL nucleus. In our cohort, RoC statistics show near

perfect separation of the PD-D group from the control subjects using MD measures of the CM/Pf. Between the PD-D and PD-N groups however, there is slightly more overlap, confirming the involvement of the CM/Pf in the motor (Henderson, et al., 2000b) as well as cognitive dysfunction of PD.

The mean diffusivity of the pulvinar nucleus was an even better discriminatory variable, separating the PD-D group from PD-N. This is in line with the grey matter loss identified in the Pu by (Beyer, Larsen, et al., 2007) and our own group (Melzer, et al., 2011b) which both showed significantly greater grey matter loss in PD-D group relative to PD-N and independent of the demographic and disease contaminants of PD.

The pulvinar may be more involved in specific subsets of dementia. In subcortical ischemic dementia patients the Pu shows reduced blood flow and a significant association with the executive function and memory subsets of cognitive dysfunction (Kato, et al., 2008). Research from a schizophrenia cohort also supports this idea, as Pu degeneration (Harms, et al., 2007) and activation (Andrews, et al., 2006) shows a strong association with tests of working memory.

The lateral posterior nucleus is also an area of significant disruption in PD-D, showing increased diffusivity relative to PD-N and healthy control subjects. Despite this, the LP nucleus showed a significant degree of overlap between PD-D and control patients and could not be considered a useful tool for identifying dementia in this cohort. This did not come as a surprise as the LP does not show extensive pathology in PD, only showing isolated LB's and LN's (Rub, Del Tredici, Schultz, et al., 2002).

The VP nucleus shows a significant increase in diffusivity in PD-D relative to control subjects and PD-N patients. The VP nucleus was also able to identify the PD-D group from the control subjects as well as from the PD-N group with some success. The VP nucleus has not previously been implicated in PD, although there is some evidence to suggest significant disruption of the primary projection site – the somatosensory cortex in PD patients who experience pain associated with dystonic symptoms (Defazio, et al., 2008). There is no evidence to suggest VP involvement in the cognitive symptoms of dementia. As the motor symptoms are more advanced in the PD-D group compared to the others in this sample however it can be assumed that VP disruption once dementia is present is in fact primarily reflective of the accompanying advance in motor dysfunction.

As expected, the motor nuclei were not influential across the cognitive changes of PD. Only the left VL nucleus shows any change from PD-N to PD-D, with increased diffusivity not evident until PD-D. Both the VA and VL separated the PD-D group from

controls and PD-N with some success however, but again this can be assumed to relate to the increased motor dysfunction in PD-D relative to the other PD groups.

8.8.4 Justification of the study methodology

For the first time, we have examined the nuclei of the thalamus simultaneously and identified dissociation between nuclei and specific domains of cognition in PD. The method used to segment the thalamic nuclei here was adapted from that of Wiegell, et al., (2003) with the application of similar methods in healthy control subjects gaining momentum in recent years. Several studies (Deoni, Rutt, Parrent, & Peters, 2007; Devlin, et al., 2006; Duan, Li, & Xi, 2007; Johansen-Berg, et al., 2005; Jonasson, et al., 2007; Rittner, Lotufo, Campbell, & Pike, 2010) have incorporated the original theory and moderated aspects of the technique in their own healthy cohorts. It is somewhat surprising therefore that this is the first time this method has been applied in Parkinson's disease, or in any neurodegenerative disorder.

The comparison between our results and those produced by Wiegell, et al., (2003) and after reference to the anatomical guidelines of several neurological atlases (Duvernoy, 1991; Niemann, et al., 2000; Nolte & Angevine, 2007) indicates that the procedure applied here has produced accurate segmentation of the thalamus for this cohort. Furthermore, given the association between specific thalamic regions and subsets of cognitive function that we have demonstrated, we believe the thalamus has undergone successful segmentation.

8.8.5 Summary

We propose that we have identified a new method to aid in the classification of cognitive dysfunction in PD. Careful monitoring of thalamic nuclei changes in PD patients will aid in identification of cognitive impairment, and could also provide neurocorrelates of cognitive dysfunction that can be monitored longitudinally.

We have confirmed the association between the LD nuclei and attention and also found an association between the CM/Pf and Pu with attention which is somewhat supported by research in other areas (Kemether, et al., 2003). We showed a previously unreported association between the VP and executive function but did not find the expected relationship with MDn and this area. In regards to learning and memory, the expected relationship between the LD and MDn was upheld but the VP produced an unexpected relationship with this domain. We expected the posterior thalamic nuclei (LP

and Pu) to be involved in visuospatial and perception but this was not upheld, and instead, the CM/Pf and LD were heavy influences here.

As hypothesised we reported that the limbic nuclei would be the first to show degeneration in PD, with the association and CM/Pf nuclei implicated soon after in PD-MCI. In accordance with other studies that studied the whole brain (Beyer & Aarsland, 2008; Summerfield, et al., 2005), the order of vulnerability follows the pattern of frontal, temporal and parietal involvement.

The thalamic nuclei have previously been examined in some neurodegenerative disease samples including in Schizophrenia (Byne, et al., 2001) and Alzheimer's disease (Braak & Braak, 1991). No studies have yet examined all thalamic nuclei simultaneously in a disease sample however. Previously, cognitive function has been associated with individual thalamic nuclei volume loss (Byne, et al., 2002), activation decreases (Andrews, et al., 2006; Buchsbaum, et al., 2006) or changes in diffusivity (Li, et al., 2010) in both disease and healthy samples but we believe we have provided extensive neuropsychological examination of a PD cohort to fully investigate cognitive function using robust statistical analysis for the first time.

Chapter 9. Study Four: THE CORTICAL CONNECTIVITY OF THE THALAMUS AND THALAMIC NUCLEI

9.1 Objectives

The white matter pathways that have been delineated from thalamic seed points are addressed in this chapter. These white matter tracts are assumed to represent the cortico-thalamic fiber network. The aim of this study was to determine the level of integrity disruption in the fiber tracts in PD and the corresponding influence of each on cognitive dysfunction. We also examined the combined influence of the thalamic tracts and the thalamic nuclei which were identified in the previous chapter. Tractography, the method used to identify fiber tracts is a new development arising from diffusion tensor imaging and has mainly only been applied in healthy samples to validate the technique. All reports which have applied tractography, in the whole brain or from individual regions are reviewed here.

9.2 Diffusion tensor fiber tracking

Just as the relationship between brain structure and function is fundamental to neuroscience (Behrens & Johansen-Berg, 2005), the relationship between brain function and connectional architecture is a fundamental principal underlying the circuits of the brain. The relationship between circuitry and function has only recently begun to be tested *in vivo* using advanced diffusion tensor methodology. Utilising the diffusion tensor image for fiber tracking is a simple principle. Just as water moves more freely through a cell unrestricted by barriers, water moves faster in tissue with a high degree of organisation. In the white matter of the brain for example, water diffusion is restricted in those voxels where the major axis of a fiber is orientated in a perpendicular, rather than parallel direction. The fiber orientation in one voxel can be systematically mapped to a colour pathway, where each colour represents the orientation of the fiber in that voxel. A path of smooth transition in colour is then able to be followed from one voxel to the next. The colour pathway can be considered to represent the trajectory of the major underlying white matter pathway (Jones, 2008).

As the principal direction of diffusion is able to be computed at each voxel, the underlying properties of fiber direction are able to be inferred based on the quantification of water movement. Using this information, large white matter tracts are able to be traced

by computing the main direction of movement in one voxel and then computing the main direction of movement in all adjacent voxels, essentially 'following' the main direction of diffusion from voxel to voxel to quantify the direction of the underlying main white matter pathways (Behrens & Johansen-Berg, 2005).

There is considerable evidence to suggest that subcortical degeneration in Parkinson's disease occurs as a result of disruption in network connectivity (Braak & del Tredici, 2008). Connectivity arising from the thalamus is particularly important as, not only is a better understanding of the connectivity pattern between the cortex and thalamus crucial for neuroscience in general (Lin, Lu, Liang, Hua, & Muzik, 2008) but it is particularly important in Parkinson's disease as the thalamico-cortico system mediates numerous symptoms of the disorder.

The thalamic white matter fiber tracts have not been explicitly examined in a Parkinson's disease cohort using a controlled study. Here, we have applied methodology modified from Behrens, et al., (2003) to investigate fiber tracts originating in each region of the thalamus. A probabilistic tractography approach is applied whereby the main direction of each voxel is determined based on several samples of the diffusion parameters at each voxel.

9.2.1 Whole brain fiber tracts

As with any new technique, fiber tracking was first applied in normal healthy control subjects. One of the first studies (Eluvathingal, Hasan, Kramer, Fletcher, & Ewing-Cobbs, 2007) applied tractography in normally developing children and adolescents to explore the normal effects of age, sex and lateralisation on cortical networks. Several tracts were isolated for analysis, mainly those connected with the frontal lobe: arcuate fasciculus (AF); inferior longitudinal fasciculus, inferior fronto-occipital fasciculus (IFOF), uncinate fasciculus (UF), corticospinal tract (CST) and the somatosensory pathway (SS). The main findings varied for each tract: there was a significant decrease in MD values of white matter pathways with age in the IFOF; a significant decrease in MD, but not FA with age for the left CST and no changes in either diffusivity measure due to age in the SS. The left hemisphere was less affected in most instances, showing higher FA values in the majority of tracts (AF, UF and CST) and lower FA only in the case of the AF.

In older samples there is a significant correlation between fiber tract integrity and cognition. Zahr (2009) investigated the fiber tracts of the corpus callosum, association cortices (cingulate, inferior longitudinal fasciculus and the uncinate) along with the fornix

in an older sample and compared the integrity with that of a younger sample. In the elderly sample, cellular disruption (lower FA and higher MD) was most prominent in the anterior tracts, especially in the genu, fornix and uncinate fibers. There was a relationship between these tracts and measures of problem solving, working memory and motor function, supporting the hypothesis that degeneration of white matter tracts in the elderly significantly contributes to age related decline.

Only one study to date has examined the integrity of the fiber tracts longitudinally. White matter tract degeneration appears to occur as a function of aging and the frontal tracts are implicated in the cognitive decline associated with Alzheimer's disease. Teipel (2010) studied 11 healthy elderly subjects and 14 subjects with amnesic MCI, performing follow up analysis at 13-16 months. The frontal white matter tracts were again heavily implicated, the superior longitudinal fasciculus, uncinate fasciculus, inferior fronto-occipital fasciculus and the cingulate bundle all exhibited decreased FA at follow up. In the MCI subjects, FA reduction was predominantly concentrated in the anterior corpus callosum. Total grey and white matter atrophy agreed with the pattern of fiber tract changes in the MCI subjects and was concentrated to prefrontal, cingulate and parietal lobe areas. Over time, the changes in fiber tracts agreed with the wider pattern of degeneration in MCI and were related to aging as well as the early stages of AD neurodegeneration in this sample.

Just as the integrity of grey matter structures has a relationship with cognition in neurodegenerative disease, the white matter tracts also strongly contribute to cognitive impairment in some samples. In Alzheimer's disease for example (Zhou, Bai, & Dougherty, 2009), the fiber pathway between the dorsal caudate area and the parahippocampal gyrus is significantly disrupted in early stage AD patients compared to control subjects. As this pathway mediates the process by which new information is integrated this suggests significant impairment in learning ability in these patients – a phenomenon known to occur in the early stages of AD (Germano & Kinsella, 2005) and underlie short term memory dysfunction.

Another AD sample (Nakata, et al., 2008) which examined the fiber integrity of the fiber tracts originating from the posterior cingulate area found significant disruption in both the direction of the fiber (FA) and the integrity of the cells (MD) within the tract. This abnormal disruption was considered to indicate a loss of barriers to diffusion and was consistent with reports of neuropathological data which showed partial loss of myelin,

axons and oligodendrial cells in the white matter of AD brains. MD and FA values in this fiber tract reflected the progression of AD related changes in the posterior cingulate tract and was considered to represent a biomarker for disease progression.

Fiber tracking techniques have also been applied in Schizophrenia (Zalesky, et al., 2010) indicating network disruption could underlie some of the behavioural symptoms such as delusions, hallucinations and disordered thoughts that are evident in this disorder. The entire cortical network was examined in these patients and significant connectivity impairment particularly evident in the medial frontal, parietal, occipital and left temporal lobe, cumulating in a 20% less efficient network in patients compared to control subjects. There was no implication for cognitive performance from this finding, although the intact network in the control subjects correlated with IQ measures.

9.2.2 Thalamic fiber tracts

Given the extensive connectivity of the thalamus with the cortex it is not surprising that the thalmic-cortico connections have also been examined in various disease samples to determine their association with dysfunction. The fronto-striatal circuit is of particular interest here due to its influence on motor dysfunction. Leh (2007) used DTI tractography and reconstructed the neural connections between the frontal cortex and the caudate nucleus and putamen in a small sample of young healthy control subjects. Results reflected what is known from animal studies, in the human brain, strong white matter pathways are evident between the caudate nucleus and the prefrontal cortex, inferior and middle temporal gyrus, frontal eye fields, cerebellum and the thalamus. The putamen is interconnected with the prefrontal, primary, supplementary and pre motor areas, the somatosensory cortex, cerebellum and the thalamus.

Lo (2011) also examined the fronto-striato-thalamic white matter tracts in healthy subjects. The main objective of this study was to determine gender and handedness differences in different white matter tracts. Three main tracts were isolated for investigation, the bilateral cingulum bundles, fornices and the anterior thalamic radiation as these are the main connections of the frontal lobe, limbic system and thalamus and mediate functions of emotion, memory and decision making. Handedness was implicated in the cingulum and anterior thalamic radiation tracts where the right tract was more impaired than the left. Gender was also a factor, showing an effect of lateralisation in male but not female subjects in the fornix tract. The authors suggested that the lateralisation by gender interaction could have been due to an increased number or greater density of axons

or myelination of the fornix in the left hemisphere that may have only been evident in the male subjects.

In bipolar disorder (Mahon, et al., 2009), tractography suggests that the thalamic radiation fibers are involved in the underlying pathology of symptoms. FA values of tracts originating from several seed points within the frontal lobes were investigated in a large sample of patients and control subjects. Tract integrity was significantly reduced in the patient group, particularly in regards to the white matter bundles that comprised the corticopontine and corticospinal tracts, pontine crossing tract and thalamic radiation fibers. The mood deregulation in bipolar disorder, could therefore, arise from deficits in cerebellar-striatal-prefrontal connectivity which encompasses structures involved in emotion generation (Green, Cahill, & Malhi, 2007). Bipolar mania is characterised by reduced frontal inhibition and could also arise due to network disruption in the frontal lobes (Foland, et al., 2008).

Fibre tracts originating from individual thalamic nuclei have been examined in few cases. Jakab (2012) was one of the first to recognise the differential influence of thalamic nuclei and segmented the mediodorsal nucleus from the rest of the thalamus using tractography and a *k* means clustering algorithm. The fibre tracts originating in the lateral portion of the MDn cluster merged with the anterior thalamic radiation and terminated in the superior and middle frontal gyri. From the medial MDn nucleus, pathways mainly joined the fronto-occipital fasciculus and the inferior longitudinal fasciculus and terminated in the frontal and temporal cortex. Although the authors reported that the MDn cluster was correlated with cognitive performance and that this was due to the fibre connectivity of the MDn to the cortex, unfortunately they did not also quantitatively examine the association between tract integrity and cognition.

Eckert and colleagues (2012) are the most recent group to publish results of thalamic fibre tracking. Fibre tracts originating from the mediodorsal and centromedian nuclei were delineated to aid in understanding of the differential connectivity of the two thalamic regions. The nuclei were preferentially associated to distinct networks, the MDn to prefrontal and limbic regions and the CM to subcortical regions. Tractography was achieved in normal healthy subjects but no examination with the correlates of cognition were carried out.

Only our own group (Melzer, et al., 2011a) has applied tractography in PD to examine the influence on cognition. A tract based spatial statistical analysis of the primary

fibre tracts across the whole brain was conducted. This study identified that the tracts arising from anterior regions of the thalamus influenced cognition. The anterior thalamic radiation, the white matter tract which connects the anterior thalamus to the frontal lobes showed significantly increased diffusivity in all three PD cognitive groups relative to control subjects, with higher values evident in the PD-D and PD-MCI groups. When analysis was limited to PD groups only however, there was no change in the anterior thalamic radiation in the PD-MCI group compared to the PD-N group, suggesting disruption in this region is not the sole influence over cognitive dysfunction in PD-MCI. Rather the anterior thalamic radiation may be a moderator of both motor and cognitive symptoms frontal symptoms in PD.

9.2.3 Fibre tracts and motor dysfunction in PD

As with other aspects of this study, the association between cortical changes and motor dysfunction cannot be discounted. In PD, some pathways have been examined specifically due to their hypothesised influence on motor dysfunction. The nigrostriatal and mesolimbic pathways were isolated in a small sample of patients with dopamine responsive PD (Gupta, et al., 2000). Although there was a significant reduction in fiber density of both tracts in PD patients, this was not investigated in relation to motor dysfunction scores. The study does highlight that diffusion measures of defined dopaminergic tracts could aid in the early diagnosis of PD and be applied as a measure of disease progression however.

The connections of the basal ganglia were also identified as a region of interest in a PD study (Nilsson, et al., 2007). Unfortunately the delineation of fiber tracts from this region was found to be impossible due to the low spatial resolution of the diffusion images. Although diffusion was measured in 32 directions, as it was in this thesis, image acquisition was achieved in the Nilsson, (2007) on a scanner of only 1.5 Telsa, rather than the 3 Telsa that was applied in our own studies. This is the most likely reason for the inability to detect the basal ganglia fiber tracts and highlights the importance of adequate spatial resolution in diffusion imaging.

9.3 Summary

Tractography has mainly been applied in healthy samples to date (Eluvathingal, et al., 2007; Teipel, et al., 2010; Zahr, et al., 2009) but there is significant evidence to suggest investigation into the integrity of network connectivity in PD is warranted. Previous

studies in PD point to significant degeneration in cortical areas associated with the thalamus (Beyer & Aarsland, 2008) and it is reasonable to expect that, alongside thalamic degeneration in corresponding areas, the white matter tracts connecting them will also be affected. Previous tractography studies in PD have focused on the integrity of the motor network and found significant degeneration in key components which influence movement symptoms (Leh, et al., 2007; Lo, et al., 2011). To date, only the tracts of the MDn and CM/Pf thalamic nuclei have been investigated using this method, and only in healthy samples (Eckert, et al., 2012; Jakab, et al., 2012). This study will be the first of its kind to address connections of major thalamic nuclei simultaneously in a Parkinson's disease sample.

9.4 Hypothesis

- That cortical connectivity between the thalamus and the cortex will be disrupted in PD and contribute to cognitive symptoms

9.5 Method

9.5.1 Participants

Participant characteristics are identical to those reported in *Section 8.6.1* as fibre tracking was achieved successfully in all participants.

9.5.2 Fibre Tracking

White matter fibre tracking was carried out on the 4D brain using the FMRIB Diffusion Toolbox (FDT: version 4.9.1) which is a software tool for the analysis of diffusion weighted images. FDT samples from the distributions of voxel-wise principal diffusion directions and computes a sample from the distribution on the location of the true direction of the sample. This process is repeated until there are many samples and the connectivity distribution is modelled. Within the toolbox, we ran two programmes. The first, Bayesian Estimation of Diffusion Parameters Obtained using Sampling Techniques (bedpostx) modelled the local diffusion parameters, building a distribution of diffusion parameters at each voxel. Bedpostx creates all the files that are necessary to run probabilistic tractography. The required inputs to the bedpostx programme is the 4D brain, the 3D brain with no diffusion weighting (nodiff_brain_mask) and the bvecs and bvalues files which contain the list of gradient directions that were applied during diffusion weighting of volumes and for the volumes with no diffusion weighting respectively. After bedpostx was applied it was possible to run the probtrackx programme. Probtrackx was used for tractography and connectivity based segmentation and gives an estimation of the most probable location of a pathway from a seed point using Bayesian techniques. The previously defined thalamic clusters were used to create a seed mask for the thalamic nuclei of each individual. For each nucleus the clusters that make up that nucleus were all able to be entered as seed points. Probabilistic tractography was then run from every voxel with a value different from 0 in this mask. The default parameters of the probtrackx algorithm were applied as follows.

9.5.2.1 Number of samples

Number of samples is the number of individual pathways that are drawn through the probability distribution. The optimum number of samples is 5000 as convergence is reached without compromising on a reasonable amount of processing time.

9.5.2.2 Steplength

Steplength determines the length of each step, where a step is the distance travelled before the direction of diffusion is re-examined. Default steplength is 0.5mm and is recommended unless the sample is of unusually small sized brains such as in the case of infants. Adjustment to steplength was not necessary as the brains in this sample are considered to be within the average range. The maximum number of steps is 2000 by default, samples were terminated when they travelled 2000 steps, corresponding to a distance of 1m at step length of 0.5mm.

9.5.2.3 Curvature threshold

Curvature threshold was 0.2, corresponding to a minimum angle of approximately ± 80 degrees. This threshold limits how sharply pathways can turn which excludes implausible pathways.

9.5.2.4 Thresholding

Once a fibre tract was constructed, the entire trajectory was verified on a slice-by-slice basis to ensure consistency with established anatomical atlases. Displayed results are original and have not been thresholded.

9.5.3 Statistical Analysis

Group differences and the relationship between tracts and cognition were examined in the same way as for the previous chapter (*Section 8.6.4*). We also wanted to examine the combined effect of nuclei and tracts in this study however so the following additional analyses were performed in Statistica version 6.0. The relationship between each thalamic nucleus and the tracts arising from the thalamic nuclei were examined first using Pearson correlation, and then using backward stepwise regression where the influence of a thalamic nucleus between every thalamic tract was determined. It was expected that the nuclei would each influence the thalamic tract that originated from that nucleus but also would have an influence on other associated tracts. Nuclei and tracts were then examined together using logistic regression analysis to aid in discrimination between the patient groups.

9.6 Results

9.6.1 Participant Information

The fibre tracking procedure was performed successfully for all participants, thus demographic, clinical and cognitive characteristics of the groups are identical to those reported in *Section 6.6.1* for the whole thalamus tracts and in *Section 8.6.1* for the thalamic nuclei tracts.

9.6.2 Integrity changes in thalamic tracts

Fibre tracts were first computed using every voxel within the thalamus as a seed point (whole thalamus tracts) to determine overall connectivity. Between – within ANOVA examined the group differences in the same way as for previous chapters. The ANCOVA models made additional provision for thalamic size due to the fact thalamic tracts varied according to the size of the thalamic nucleus. Total thalamic volume (uncorrected in mm^3) was therefore the first co-variate added to the ANCOVA model, followed by demographic and clinical co-variates in subsequent models in the same way as before. Group mean values of DTI parameters for whole thalamus tracts are presented in *Table 9-1* and adjacent pair wise comparisons after accounting for co-variates in *Figure 9-1*. Unexpectedly, there was no group effect for FA values of thalamic tracts. There was a hemisphere effect in the left > right direction but this did not result in a group x hemisphere interaction. Results did not change after the addition of thalamic volume, [$F(3,112) = 1.83$, $p = 0.32$]; demographic, [$F(3,109) = 1.32$, $p = 0.27$] or clinical [$F(2,84) = 1.44$, $p = 0.24$] covariates.

MD of thalamic tracts was more sensitive to the changes between cognitive groups. There was a significant effect of group where MD was higher in the PD-D group relative to all other groups. The group effect was consistent for both the left and the right tracts; there was no effect of hemisphere or higher order interactions. The group effect remained when total thalamic volume was included in the model [$F(3,112) = 8.17$, $p < 0.01$]. Once thalamic size was accounted for, the additional post hoc contrast between PD-MCI and the control group was also significant in the expected direction. This contrast, and the overall group effect remained after the addition of demographic [$F(3,109) = 5.74$, $p < 0.01$] but not clinical covariates [$F(2,84) = 2.13$, $p = 0.13$].

Table 9-1: Diffusion parameters of averaged bilateral thalamo – cortical fiber tracts

	C (n = 25)	PD-N (n = 56)	PD-MCI (n = 19)	PD-D (n = 17)	Group Effect			Hemisphere Effect	
					F	p	N-K	F	p
Fractional Anisotropy (range 0-1)	**0.41 (0.02)**	**0.41 (0.02)**	**0.40 (0.02)**	**0.40 (0.04)**	**1.43**	**0.24**	**C=N=MCI=D**	**4.02**	**0.05**
Left	0.41 (0.02)	0.41 (0.03)	0.40 (0.02)	0.41 (0.03)	1.23	0.30			
Right	0.41 (0.02)	0.41 (0.03)	0.40 (0.02)	0.40 (0.03)	1.46	0.23			
Mean Diffusivity (mm^2 per sec x 10^{-3})	**0.57 (0.28)**	**0.66 (0.28)**	**0.79 (0.31)**	**0.98 (0.24)**	**8.35**	**<.001**	**C=N=MCI<D**	**0.77**	**0.38**
Left	0.57 (0.27)	0.66 (0.28)	0.79 (0.31)	0.98 (0.25)	8.23	<.001	C=N=MCI<D		
Right	0.57 (0.28)	0.66 (0.29)	0.80 (0.31)	0.98 (0.23)	8.36	<.001	C=N=MCI<D		

Fractional anisotropy and mean diffusivity values for each group are reported mean (SD). Fiber tracking was able to be performed on all participants where the thalamus was able to be defined, so sample size here reflects that of *Section 6.7.1*. Between-within ANOVA where group was the between effect and hemisphere the within effect shows the group and hemisphere effect for FA and MD measure of bilateral thalamus (**bold** face). The hemisphere effect for FA is in the left > right direction. There were no higher order interactions. **N-K** = Newman-Keulis post-hoc test.

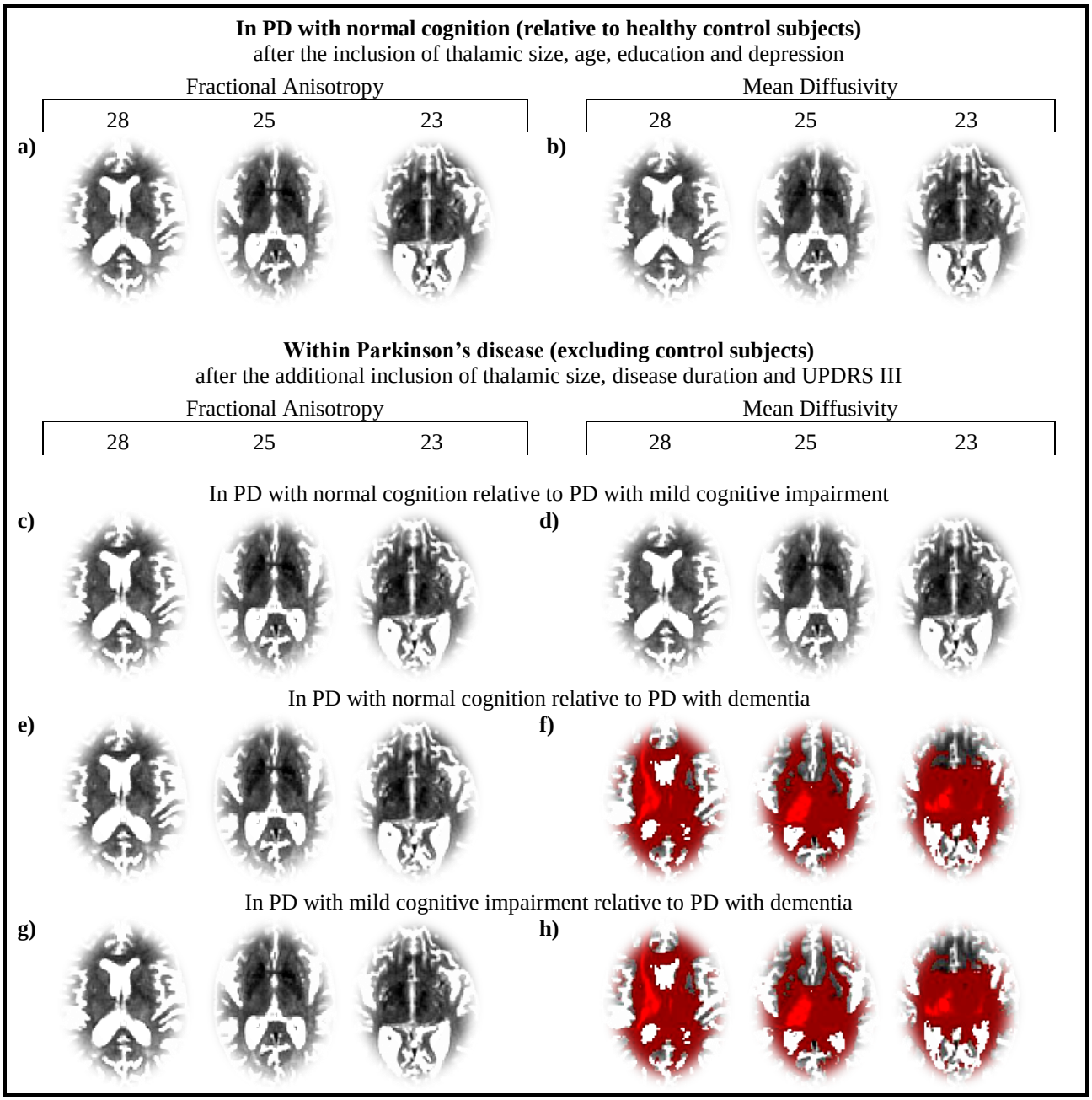


Figure 9-1: The progressive changes in whole thalamus tracts
Horizontal sections of the furtherest dorsal (Z = 28), medial (Z = 25) and furtherest ventral (Z = 23) slice where the thalamus is visible are presented. The fibre tracts have been generated from all thalamus voxels. Progressive FA (left) and MD (right) changes in whole thalamus tracts are presented from control subjects to PD-N, from PD-N to PD-MCI ($p < 0.001$) and from PD-MCI to PD-D ($p = 0.03$). The additional comparison where the PD-N group is used as a control group to establish changes in the PD-D group is also presented in the middle row.

Fibre tracts were then examined independently for each thalamic nucleus (nuclei tracts) to determine the integrity of differential connectivity. For FA measures of integrity (*Table 9-2*), there was clear differential involvement of the thalamic nuclei; only the limbic nuclei tracts were sensitive to cognition. In both the AP and LD nuclei tracts there was an overall significant group effect and a significant post hoc effect where the PD-D group

showed reduced FA compared to both the control and PD-N groups. There was also a significant group by hemisphere interaction [$F(3,104) = 4.74$, $p < 0.01$] in the AP nucleus which warranted follow up analyses. One way ANOVA's (indented), conduced independently for left and right hemisphere of the AP tract revealed the group effect to be driven by the left hemisphere, the PD-N group had lower FA than the control group ($p < 0.01$) and the PD-D group had lower FA than the PD-N group ($p < 0.01$) but only in the right hemisphere did the PD-D group show reduced FA in comparison to the control group ($p = 0.04$).

Including AP size [$F(3,103) = 7.23$, $p < 0.01$] and demographic [$F(3,100) = 4.63$, $p < 0.01$] covariates did not change the overall group effect although the post-hoc results changed, the PD-D group only had reduced FA compared to the PD-N group ($p < 0.01$) but not the control group ($p = 0.05$). As the interaction between hemisphere and group [$F(3,100) = 3.56$, $p = 0.02$] remained when AP size was included along with demographic covariates, independent follow up analyses were again conducted for left and right AP. The group effect again only held in the left, [$F(3,100) = 7.38$, $p < 0.01$] but not the right, [$F(3,100) = 2.21$, $p = 0.09$] AP. After the addition of clinical covariates the overall group effect remained [$F(2,75) = 3.97$, $p = 0.02$] and the interaction between group and hemisphere [$F(2,75) = 1.03$, $p = 0.36$] was no longer significant.

In the LD nucleus tract FA was decreased in PD-D relative to all other groups. Although there was an effect of hemisphere, where the left tract showed higher FA than the right, this was consistent for all groups and there was not a higher order interaction between group and hemisphere. Adding LD size [$F(3,100) = 3.16$, $p = 0.03$] and demographic [$F(3,101) = 3.11$, $p = 0.03$] covariates did not change results but the group effect was no longer significant when clinical covariates were added [$F(2,75) = 2.31$, $p = 0.11$].

Table 9-2: FA (range 0-1) values for each thalamic nuclei – cortical fiber tract

	C (n=24)	PD-N (n=51)	PD-MCI (n=18)	PD-D (n=15)	Group			Hemisphere	
					F	p	N-K	F	p
Association Nuclei									
MDn	**0.37 (0.03)**	**0.38 (0.03)**	**0.37 (0.02)**	**0.36 (0.04)**	**1.59**	**0.20**		**7.35**	**<0.01**
Left	0.38 (0.04)	0.38 (0.03)	0.38 (0.03)	0.37 (0.05)	0.43	0.73			
Right	0.36 (0.03)	0.37 (0.04)	0.36 (0.03)	0.35 (0.05)	1.53	0.21			
LP	**0.42 (0.04)**	**0.42 (0.04)**	**0.41 (0.04)**	**0.42 (0.05)**	**0.60**	**0.62**		**4.67**	**0.03**
Left	0.43 (0.04)	0.43 (0.05)	0.41 (0.05)	0.43 (0.05)	0.50	0.68			
Right	0.42 (0.06)	0.41 (0.05)	0.40 (0.04)	0.41 (0.07)	0.61	0.61			
Pu	**0.41 (0.04)**	**0.42 (0.04)**	**0.40 (0.03)**	**0.40 (0.04)**	**1.66**	**0.18**		**0.06**	**0.81**
Left	0.42 (0.05)	0.42 (0.05)	0.39 (0.05)	0.39 (0.04)	2.57	0.06			
Right	0.40 (0.05)	0.42 (0.05)	0.41 (0.06)	0.41 (0.06)	0.85	0.42			
Limbic Nuclei									
AP	**0.36 (0.03)**	**0.37 (0.03)**	**0.35 (0.03)**	**0.34 (0.03)**	**5.18**	**<0.01**	**C=N>D**	**5.25**	**0.02**[I]
Left	0.35 (0.03)	0.38 (0.03)	0.36 (0.03)	0.34 (0.03)	8.85	<0.01	C>N=MCI=D		
Right	0.36 (0.03)	0.36 (0.04)	0.34 (0.04)	0.33 (0.04)	2.74	0.05	C>D		
LD	**0.34 (0.04)**	**0.35 (0.04)**	**0.33 (0.04)**	**0.31 (0.03)**	**5.12**	**<0.01**	**C=N=MCI>D**	**23.07**	**<0.001**
Left	0.36 (0.06)	0.36 (0.06)	0.35 (0.06)	0.33 (0.06)	1.13	0.34			
Right	0.32 (0.04)	0.34 (0.05)	0.32 (0.06)	0.28 (0.04)	5.67	<0.01			
Sensory Nucleus									
VP	**0.45 (0.04)**	**0.45 (0.03)**	**0.45 (0.03)**	**0.45 (0.03)**	**0.42**	**0.74**		**0.82**	**0.37**
Left	0.45 (0.04)	0.45 (0.04)	0.45 (0.03)	0.46 (0.03)	0.38	0.77			
Right	0.45 (0.04)	0.45 (0.03)	0.44 (0.03)	0.44 (0.04)	0.81	0.49			
Motor Nuclei									
VA	**0.42 (0.04)**	**0.43 (0.03)**	**0.41 (0.03)**	**0.41 (0.04)**	**2.90**	**0.04**		**7.13**	**<0.01**
Left	0.42 (0.04)	0.44 (0.04)	0.42 (0.04)	0.41 (0.04)	3.07	0.03			
Right	0.41 (0.04)	0.42 (0.03)	0.41 (0.04)	0.40 (0.05)	1.54	0.21			
VL	**0.44 (0.04)**	**0.44 (0.03)**	**0.43 (0.03)**	**0.43 (0.03)**	**0.17**	**0.92**		**0.63**	**0.43**
Left	0.43 (0.04)	0.44 (0.04)	0.43 (0.03)	0.44 (0.03)	0.41	0.75			
Right	0.44 (0.04)	0.43 (0.03)	0.43 (0.03)	0.43 (0.03)	0.16	0.93			
Non-Specific Nucleus									
CM/Pf	**0.40 (0.04)**	**0.40 (0.04)**	**0.39 (0.04)**	**0.40 (0.04)**	**0.23**	**0.87**		**0.82**	**0.37**
Left	0.41 (0.05)	0.40 (0.05)	0.39 (0.05)	0.40 (0.04)	0.22	0.88			
Right	0.39 (0.04)	0.40 (0.06)	0.39 (0.04)	0.40 (0.06)	0.20	0.90			

Fractional anisotropy values for each thalamic nucleus are reported mean (SD). Repeated measures ANOVA (bold face) shows the group and hemisphere effect for each nucleus. All hemisphere effects were in the L>R direction. [I] Denotes an interaction between group and hemisphere, follow up one way ANOVA's (indented) show the group effect independently of hemisphere. All significant post-hoc group comparisons are reported, not only adjacent comparisons. **N-K** = Newman-Keulis.

MD measures of thalamic tracts were more sensitive to the changes between cognitive groups than FA measures (*Table 9-3*). All nuclei tracts except those originating from the CM/Pf, LP and Pu nuclei showed a main effect of group. Post hoc comparisons showed a significant increase in the PD-D group compared to the control group in the AP ($p < 0.01$); MDn ($p < 0.01$); VA ($p < 0.001$); VL ($p = 0.016$); VP ($p = 0.03$) nuclei tracts and, compared to the PD-N group in the AP ($p < 0.001$); MDn ($p < 0.01$); LD ($p < 0.01$); VA ($p < 0.001$); VL ($p = 0.01$) and VP ($p = 0.01$) nuclei tracts. In the MDn ($p < 0.01$); AP ($p < 0.01$); and VA ($p = 0.03$) tracts there was an additional significant increase in PD-D compared to the PD-MCI group. The VA ($p = 0.05$) was the only region to show a significant increase from PD-N to PD-MCI.

Although there was not a hemisphere effect in the thalamic tract that was delineated from the whole thalamus, all individual nuclei tracts had higher MD values in the left tract compared to the right. There was a significant group by hemisphere interaction in the tract of the AP nucleus [$F(3,104) = 4.50$, $p < 0.01$] which suggested the need for further analyses independent of hemisphere. Follow up one way ANOVA's for the AP tract (indented) showed that it was again the left hemisphere that was more sensitive to lower levels of cognitive dysfunction. Although there was an overall significant group effect in both hemispheres and a significant post hoc comparison between PD-D and both the control (left $p < 0.01$; right $p < 0.01$) and the PD-N (left $p < 0.001$; right $p = 0.03$), only the left hemisphere showed an increase in diffusivity from PD-MCI to PD-D ($p < 0.001$).

Table 9-3: MD (mm^2 per sec $x10^{-3}$) values of each thalamic nucleus – cortical fiber tract

	C (n=23)	PD-N (n=51)	PD-MCI (n=18)	PD-D (n=15)	Group			Hemisphere	
					F	p	N-K	F	p
Association Nuclei									
MDn	**0.93 (0.07)**	**0.95 (0.11)**	**0.95 (0.08)**	**1.04 (0.09)**	**4.12**	**<0.01**	**C=N=MCI<D**	**0.13**	**<0.01**
Left	0.93 (0.08)	0.95 (0.13)	0.96 (0.10)	1.01 (0.11)	1.60	0.19			
Right	0.93 (0.08)	0.94 (0.12)	0.95 (0.10)	1.06 (0.09)	5.27	<0.01	C=N=MCI<D		
LP	**0.92 (0.07)**	**0.93 (0.11)**	**0.96 (0.08)**	**0.96 (0.08)**	**0.75**	**0.53**	**C=N<D**	**0.04**	**0.85**
Left	0.95 (0.09)	0.93 (0.13)	0.96 (0.13)	0.95 (0.04)	0.51	0.68			
Right	0.91 (0.10)	0.94 (0.14)	0.95 (0.07)	0.98 (0.14)	1.12	0.34			
Pu	**0.96 (0.09)**	**0.93 (0.11)**	**1.00 (0.10)**	**1.01 (0.07)**	**3.67**	**0.01**		**0.05**	**0.82**
Left	0.95 (0.10)	0.93 (0.13)	1.00 (0.13)	1.03 (0.08)	3.90	0.01	C=N<D		
Right	0.98 (0.12)	0.93 (0.13)	1.00 (0.14)	0.99 (0.10)	1.76	0.16			
Limbic Nuclei									
AP	**0.98 (0.08)**	**0.97 (0.12)**	**0.99 (0.09)**	**1.10 (0.09)**	**5.75**	**<0.01**	**C=N=MCI<D**	**0.01**	**0.92[I]**
Left	1.00 (0.09)	0.95 (0.14)	0.97 (0.08)	1.11 (0.10)	7.04	<.001	C=N=MCI<D		
Right	1.00 (0.09)	0.99 (0.15)	1.02 (0.16)	1.09 (0.10)	3.82	0.01	C=N>D		
LD	**1.14 (0.16)**	**1.09 (0.19)**	**1.18 (0.17)**	**1.27 (0.12)**	**4.75**	**<0.01**	**C=N<MCI=D**	**8.73**	**<0.01**
Left	1.10 (0.03)	1.06 (0.24)	1.15 (0.21)	1.16 (0.17)	1.17	0.33			
Right K-W ANOVA	1.19 (0.19)	1.11 (0.23)	1.21 (0.34)	1.37 (0.21)	12.3	<0.01	C=N<D		
Sensory Nucleus									
VP	**0.83 (0.03)**	**0.84 (0.07)**	**0.87 (0.06)**	**0.89 (0.05)**	**3.81**	**0.01**	**C=N<D**	**1.65**	**0.20**
Left	0.84 (0.03)	0.84 (0.13)	0.88 (0.06)	0.88 (0.04)	4.20	<0.01	C=N<MCI=D		
Right	0.83 (0.04)	0.83 (0.09)	0.85 (0.05)	0.88 (0.06)	2.79	0.04	C=N<D		
Motor Nuclei									
VA	**0.84 (0.06)**	**0.83 (0.08)**	**0.88 (0.08)**	**0.93 (0.06)**	**8.73**	**<.001**	**C=N<MCI<D**	**10.56**	**<0.01**
Left	0.84 (0.06)	0.81 (0.07)	0.87 (0.08)	0.91 (0.06)	7.83	<.001	C=N<MCI=D		
Right	0.84 (0.05)	0.84 (0.09)	0.88 (0.08)	0.94 (0.08)	8.08	<.001	C=N=MCI<D		
VL	**0.82 (0.04)**	**0.82 (0.08)**	**0.85 (0.05)**	**0.88 (0.05)**	**3.98**	**<0.01**	**C=N<D**	**3.98**	**<0.01**
Left	0.83 (0.04)	0.83 (0.09)	0.86 (0.06)	0.88 (0.05)	2.57	0.06			
Right	0.82 (0.05)	0.82 (0.07)	0.85 (0.05)	0.88 (0.04)	4.81	<0.01	C=N<D		
Non-Specific Nucleus									
CM/Pf	**0.88 (0.06)**	**0.88 (0.10)**	**0.91 (0.08)**	**0.94 (0.05)**	**2.31**	**0.08**		**0.27**	**0.61**
Left	0.88 (0.08)	0.87 (0.10)	0.90 (0.09)	0.94 (0.06)	2.75	0.05	C=N<D		
Right	0.88 (0.07)	0.89 (0.13)	0.91 (0.08)	0.94 (0.07)	1.01	0.39			

Mean diffusivity values for each thalamic nucleus are reported mean (SD). Repeated measures ANOVA (**bold** face) shows the group and hemisphere effect for each nucleus. All hemisphere effects were in the L>R direction. [I] Denotes an interaction between group and hemisphere, follow up one way ANOVA's (indented) show the group effect independently of hemisphere. All significant post-hoc group comparisons are reported, not only adjacent comparisons. **N-K** = Newman-Keulis

After the inclusion of nuclei size and demographic covariates (*Table 9-4*). There was no longer a group effect of MD in any thalamic nuclei tract except the VA. This did not change when control participants were excluded and clinical covariates included. The interaction between hemisphere and group remained in the AP nucleus after inclusion of demographic covariates [$F(3,100) = 3.69$, $p = 0.01$], and as the overall group effect was approaching significance follow up analyses were conducted for this region. The left hemisphere was again the most sensitive, only MD measures of the left thalamic nuclei tract had a group effect, there was no effect of cognition in the right AP tract.

Table 9-4: MD ANCOVA results after inclusion of demographic and clinical covariates

	Demographic (All participants) Nuclei size, age, education, depression			Clinical (Patients only) adds disease duration, UPDRS III		
	Group Effect			Group Effect		
	F(3,100)	p	Adjacent pairwise	F(2,75)	p	Adjacent pairwise
Association Nuclei						
MDn	1.55	0.21				
LP	0.11	0.96				
Limbic Nuclei						
AP	2.68	0.05				
Left	3.96	0.01	C=N=MCI<D	4.36	0.02	N<MCI<D
Right	1.88	0.14		0.43	0.65	
LD	1.99	0.12				
Sensory Nucleus						
VP	0.82	0.49				
Motor Nucleus						
VA	3.26	0.02	C=N<MCI<D	5.17	0.02	N<MCI<D
VL	1.23	0.30				

Nuclei tracts that previously indicated a group effect of mean diffusivity were examined using ANCOVA's where nuclei size along with demographic and clinical covariates were added. After inclusion of demographic and clinical covariates the overall group effect only remained significant in the VA tract. As there was an interaction between group and hemisphere in the AP tract, independent one way ANOVA's were also conducted on the left and right tract (indented).

Post-hoc pairwise comparisons after the inclusion of covariates are shown in *Figure 9-2* for both FA and MD measures of thalamic nuclei tracts. Tracts were examined separately within each hemisphere as the left hemisphere has consistently demonstrated more sensitivity to cognitive dysfunction in this cohort.

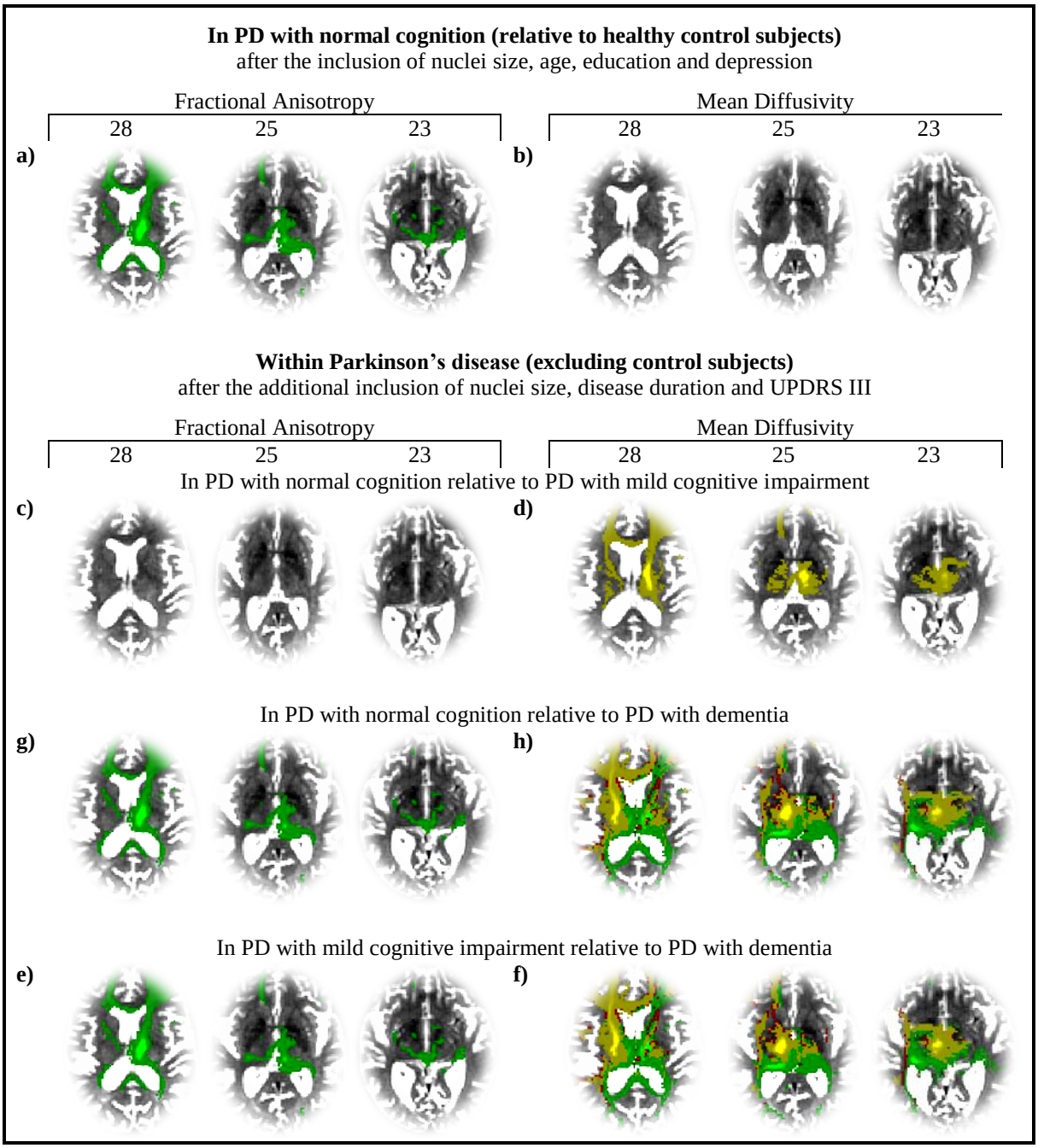


Figure 9-2: Adjacent pairwise comparisons in nuclei tracts
Horizontal sections of the furtherest dorsal (Z = 28), medial (Z = 25) and furtherest ventral (Z = 23) slice where the thalamus is visible are presented. The fibre tracts have been generated from nuclei voxels only. Progressive FA (left) and MD (right) changes are presented from control subjects to PD-N, from PD-N to PD-MCI and from PD-MCI to PD-D. The additional comparison where the PD-N group is used as a control group to establish changes in the PD-D group is also presented. Statistics are reported within the text. Fiber tracts originating from the motor nuclei (VA) are shown in yellow, limbic nuclei (AP, LD) in green and association (MDn) nuclei in red.

After controlling for co-variates, fractional ainsotropy measures were only sensitive to tract degeneration in the AP tract of the left hemisphere [$F(3,100) = 7.13$, $p < 0.001$]. FA reduction was evident in PD-N relative to the healthy control group ($p < 0.01$) and also

significantly disrupted in PD-D relative to PD-N ($p < 0.01$) and PD-MCI ($p = 0.03$), suggesting a significant association with cognitive dysfunction.

Mean diffusivity measures of thalamic nuclei tracts also implicated the left AP [$F(2,75) = 4.36$, $p = 0.02$] in PD-D relative to PD-N ($p < 0.001$) and PD-MCI (left $p < 0.001$) but not in PD-N relative to controls, suggesting primary involvement in cognitive, rather than motor dysfunction. The other limbic nucleus, the LD also showed significant cellular disruption in the PD groups with worse cognition [$F(2,75) = 3.90$, $p = 0.02$]. This was localised to the right hemisphere and MD was increased in PD-D relative to PD-N ($p < 0.01$), and PD-MCI ($p = 0.04$). The only other tract known to associate with cognition that was implicated was the association MDn nucleus tract which showed significant MD increase in the right hemisphere [$F(2,75) = 4.85$, $p = 0.01$] in PD-D relative to PD-N ($p < 0.01$) and PD-MCI ($p = 0.001$). The motor VA nucleus tract also showed significant degeneration, in PD-MCI relative to PD-N on the left [$F(2,75) = 4.82$, $p = 0.02$], in PD-D relative to PD-N bilaterally (left $p < 0.001$; right $p < 0.001$) and in PD-D relative to PD-MCI on the right ($p < 0.01$) which suggested motor symptoms needed to be accounted for when examining the association with cognition.

9.6.3 The association between the integrity of thalamic tracts and cognition

9.6.3.1 Correlation results

A simple Pearson correlation was used to ascertain the relationship between the integrity measures of thalamic tracts and cognition. In all cases left and right tracts were averaged and the new average variable entered against all cognitive domain scores (attention, executive function, visuospatial/visuoperception, learning and memory and global Z score).

In healthy controls

In healthy control subjects there was no association between the integrity measures of whole thalamus tracts and cognition. When the integrity of tracts was examined from each thalamic nucleus however there was an association with some individual nuclei tracts. Irrespective of the measure of integrity (FA or MD), only the association nuclei had a significant relationship with cognition. FA measures showed a relationship between the MDn nucleus and: attention ($r = 0.43$, $p = 0.04$); executive function ($r = 0.43$, $p = 0.04$) and between the LP nucleus and attention ($r = 0.42$, $p = 0.04$). When measured with MD, results were similar; the MDn nucleus was correlated with learning and memory ($r = -0.41$, $p = 0.04$) and the LP was correlated with attention ($r = -0.42$, $p = 0.04$).

In Parkinson's disease

Correlation results for Parkinson's disease patients are presented in *Table 9-5*. There was no relationship between FA measures of whole thalamic tracts and any cognitive domain. Mean diffusivity measures were more sensitive to cognitive dysfunction and showed a relationship with all cognitive domains. When examined by specific thalamic nuclei the MDn and LP association nuclei and the AP limbic nucleus had a relationship with all cognitive domains. The limbic LD nucleus was also heavily involved in cognition, showing a relationship with all domains except the visuospatial/perception domain. Unexpectedly, the motor nuclei also had an association with multiple cognitive domains.

Table 9-5: Pearson correlations (*r*) between thalamic nuclei tracts and cognition in PD patients

	Attention	Executive Function	Visuospatial	Learning and Memory	Global Z Score
Fractional Anisotropy					
Whole thalamus					
Average	-0.03	-0.05	-0.56	-0.03	-0.04
Association nuclei					
MDn	0.18	0.16	0.14	0.25*	0.21
LP	0.04	0.03	0.06	-0.03	0.03
Pu	0.18	0.16	0.16	0.07	0.16
Limbic nuclei					
AP	0.33*	0.28**	0.17	0.26*	0.30**
LD	0.42***	0.36***	0.32**	0.19	0.36***
Sensory nucleus					
VP	0.01	-0.03	0.05	0.11	0.06
Motor nuclei					
VA	0.26*	0.24*	0.20	0.16	0.24*
VL	0.04	0.01	-0.05	0.02	0.13
Non-specific nuclei					
CM/Pf	-0.18	-0.08	-0.12	-0.10	-0.06
Mean Diffusivity					
Whole thalamus					
Average	-0.32**	-0.31**	-0.27*	-0.28**	-0.33**
Association nuclei					
MDn	-0.30**	-0.27*	-0.29***	-0.28**	-0.33**
LP	-0.12	-0.12	-0.13	-0.07	-0.12
Pu	-0.36***	-0.34***	-0.34***	-0.27*	-0.34***
Limbic nuclei					
AP	-0.31*	-0.30**	-0.21	-0.26*	-0.31**
LD	-0.39***	-0.34**	-0.31**	-0.23*	-0.36***
Sensory nucleus					
VP	-0.32***	-0.30***	-0.24*	-0.26*	-0.31***
Motor nuclei					
VA	-0.45***	-0.41***	-0.34***	-0.39***	-0.44***
VL	-0.34*	-0.30***	-0.19	-0.26*	-0.30**
Non-specific					
CM/Pf	-0.22*	-0.16	-0.13	-0.20	-0.20

The relationship between FA and MD measures of thalamic nuclei tracts and each cognitive domain were performed for PD patients only. Significant correlations are indicated as follows: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$.

9.6.3.2 Regression modelling results

Regression analysis were conducted to determine the independent influence of the thalamic tracts over cognition. In order to adhere to the assumptions of multiple regression, only those tracts which showed a significant correlation with cognitive domain (*Table 9-5*) were included. Control subjects were also excluded from the analysis in order for the clinical covariates to be included. Due to violation of the multicolinearity assumption the UPDRS III score was excluded and only disease duration remained in the model as a measure of clinical severity of motor symptoms. Two backward stepwise models were performed.

The first examined the independent effect of thalamic nuclei tracts on each cognitive domain. The second repeated the parameters of the first model but additionally included demographic and clinical covariates in order to determine the influence of tracts over and above the demographic and clinical factors.

Fractional anisotropy

Results for fractional anisotropy measures of thalamic nuclei tracts are presented in *Table 9-6.* The first model for each cognitive domain was significant, indicating that combined, FA measures of integrity made a significant contribution to cognitive score in each domain. Within each of these models, the limbic nuclei were the only unique predictors that were involved in every cognitive domain, which indicated they were the only regions to independently contribute to all aspects of cognition. When the covariates were added in Model 2 the proportion of variance accounted for in each cognitive domain increased. Neither of the limbic nuclei independently contributed to the variance in the learning and memory or global Z score domain in these models but continued to contribute to the variance in attention, executive function and visuospatial/perception.

Table 9-6: Backward stepwise linear regression results for FA of nuclei tracts

% of variance accounted for by unique predictors				R2 change
Model 1 Nuclei tracts (specified for each model)		Model 2 Nuclei tracts and covariates age, education, depression, disease duration		
Attention				
Entered tracts: AP, LD, VA				
R^2 = 20%, F(3,80) = 6.37, p<0.001		R^2 = 44%, F(7,76) = 8.36, p<0.001		↑ 19% (p<0.01)
LD (β = 0.32*)	5% (0.04)	LD (β = 0.23*)	3% (0.04)	
		Age (β = -0.21*)	4% (0.03)	
		Depression (β = -0.22*)	4% (0.04)	
		Disease duration (β = -0.23*)	4% (0.02)	
Executive Function				
Entered tracts: AP, LD, VA				
R^2 = 14%, F(3,80) = 4.36, p<0.001		R^2 = 44%, F(7,76) = 8.36, p<0.001		↑ 29% (p<0.01)
LD (β = 0.27*)		LD (β = 0.17)	3% (0.04)	
		Age (β = -0.20*)	6% (<0.01)	
		Education (β = 0.22*)	4% (0.02)	
		Depression (β = -0.30***)	8% (<0.01)	
		Disease duration (β = -0.32***)	8% (<0.01)	
Visuospatial / Perception				
Entered tracts: LD				
R^2 = 10%, F(1,82) = 9.12, p<0.01		R^2 = 25%, F(5,78) = 5.32, p<0.01		↑ 15% (p<0.01)
LD (β = 0.32**)	10% (<0.01)	LD (β = 0.19)	5% (0.03)	
		Disease Duration (β=-0.32*)	6% (0.01)	
Learning and Memory				
Entered tracts: AP, MD				
R^2 = 9%, F(2,81) = 4.08, p=0.02		R^2 = 27%, F(6,77) = 4.65, p<0.01		↑ 17% (p<0.01)
AP (β = 0.19)	7% (0.02)	Age (β = -0.22*)	9 (<0.01)	
		Depression (β=-0.28**)	9 (<0.01)	
Global Z score				
Entered tracts: AP, LD, VA				
R^2 = 15%, F(3,80) = 4.56, p<0.01		R^2 = 39%, F(7,76) = 6.94, p<0.01		↑ 24% (p<0.01)
LD (β = 0.26*)	4% (0.04)	Age (β = -0.22*)	8% (<0.01)	
		Education (β = 0.57*)	4% (0.04)	
		Depression (β = -0.26**)	6% (0.01)	
		Disease duration (β=-0.27**)	6 (<0.01)	

Two linear models were used to gauge the proportion of variance accounted for by FA measures of thalamic nuclei tracts in each cognitive domain. Only those nuclei that had a significant correlation with the cognitive domain were entered into the model. Only the limbic nuclei tracts were independent predictors of cognition. R^2 = proportion of variance accounted for. Significant Beta values are indicated as follows: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$.

Mean diffusivity

Results for mean diffusivity measures of thalamic nuclei tracts are presented in *Table 9-7.* All of the first Model 1 analyses were significant, indicating that combined, the effect of the nuclei tracts that were entered into the model accounted for a significant proportion of

the variance in cognitive domain scores, for all cognitive domains. Within Model 1, the only independent predictor of any cognitive domain was the motor VA nucleus which accounted for a significant independent proportion of the variance in attention, executive function, learning and memory and global Z score. As expected, after the inclusion of covariates in Model 2, there was an increase in the proportion of variance accounted for in all cognitive domain scores. Within Model 2, the VA nucleus remained an independent predictor of the attention and global Z score domains and became an independent predictor of the visuospatial domain.

Table 9-7: **Backward stepwise linear regression results for MD of nuclei tracts**

% of variance accounted for by unique predictors				R2 change
Model 1		**Model 2**		
Nuclei tracts (specified for each model)		Nuclei tracts and covariates age, education, depression, disease duration		
Attention				
Entered tracts: AP, MDn, CM/Pf, LD, VA, VL, VP, Pu				
R^2 = 27%, F(8,75) = 3.45, p<0.01		R^2 = 43%, F(12,71) = 4.49, p<0.001		↑16%
VA (β = -0.67**)	7% (<0.01)	VA (β = -0.54*)	4% (0.03)	(p<0.001)
		Disease Duration (β=-0.30**)	8 (<0.01)	
Executive Function				
Entered tracts: AP, MDn, LD, VA, VL, VP, Pu				
R^2 = 20%, F(7,76) = 2.72, p<0.01		R^2 = 48%,F(11,72) = 6.0, p<0.001		↑28%
VA (β = -0.53*)	4% (0.04)	Education (β = 0.24*)	4% (0.02)	(p<0.001)
		Depression (β = -0.29**)	7 % (<0.01)	
		Disease Duration (β = -0.37†)	12% (<0.001)	
Learning and Memory				
Entered tracts: AP, MDn, LD, VA, VL, VP, Pu				
R^2 = 17%, F(7,76) = 2.21, p=0.04		R^2 = 30%, F(11,72) = 2.82, p<0.01		↑13
VA (β= -0.64*)	6% (0.02)	Depression (β = -0.25*)	6% (0.02)	(p=0.01)
		Disease Duration (β= -0.20)	5% (0.03)	
Visuospatial/Visuoperception				
Entered tracts: MDn, LD, VA, VP, Pu				
R^2 = 16%, F(5,78) = 2.82, p<0.02		R^2 = 28%, F(9,74) = 3.28, p<0.01		↑13%
none		VA (β = -0.26)	8% (0.01)	(p=0.01)
		Disease Duration (β=-0.30*)	8% (<0.01)	
Global Z Score				
Entered tracts: AP, MDn, LD, VA, VL, VP, Pu				
R^2 = 24%, F(7,76) = 3.41, p<0.01		R^2 = 44%, F(11,72) = 5.15, p<0.001		↑ 20%
VA (β= -0.62*)	6 % (0.02)	VA (β = -0.48*)	3% (0.05)	(p<0.001)
		Education (β = 0.20*)	4% (0.03)	
		Depression (β = -0.24*)	5% (0.01)	
		Disease Duration (β=-0.32***)	9% (<0.001)	

Two linear models were used to analyse the proportion of variance accounted for by MD measures of thalamic nuclei tracts in each cognitive domain. Only those nuclei that had a significant correlation with the cognitive domain were entered into the model. Only the motor nuclei tract was an independent predictor of cognition. R^2 = proportion of variance accounted for. Significant Beta values are indicated as follows: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$.

9.6.4 Fibre tracts as a tool for group discrimination

Receiver operating curve (RoC) statistics were generated independently from FA and MD measures of tract integrity to calculate the predictive validity of each thalamic nuclei tract and are reported in *Table 9-8.* Several pairwise comparisons were conducted to first separate the patient group with no cognitive impairment from healthy control participants and then to separate mild cognitive impairment and dementia from both the healthy controls and the unimpaired PD-N group.

9.6.4.1 Identification of the patient group

The PD-N group could not be separated from the healthy controls; in every case the AUC of each thalamic nuclei tract was not significant.

9.6.4.2 Identification of dementia

Identification of the PD-D group relative to the other groups was not achieved as easily as it was using diffusion measures of the nuclei in the previous chapter. The comparison between PD-D and controls and the PD-N group was best achieved using sensory and motor nuclei tracts whilst the discrimination between PD-D and PD-MCI was best achieved using the association tracts and the limbic AP tract. Relative to the control subjects the best discriminatory variable was the sensory VP nucleus, but this only allowed for identification of PD-D 66.7% of the time. Identification of PD-D was achieved with higher success when discriminating from the PD-N group, MD of the VA identified PD-D patients 86.7% of the time. Not surprisingly, separating the PD-D group from the PD-MCI group proved more difficult. MD values of tracts gave higher AUC than FA in most cases, but for the LP and LD nuclei tracts FA was the better diffusion measure. In terms of the accuracy of the separation, only MD measures of the limbic AP tract allowed for correct identification of the PD-D group at a level greater than chance (66.67%).

9.6.4.3 Identification of mild cognitive impairment

Separating the PD-MCI group from the controls was not successful, there were no thalamic nuclei tracts that gave a significant area under the curve. Although MD measures of the association (Pu), limbic (LD) and motor (VA) nuclei tracts all had a significant AUC, none of these regions enabled successful discrimination between the two groups with a great degree of accuracy, all showed sensitivity less than 50%.

Table 9-8: Predicting PD and PD cognitive impairment using DTI measures of nuclei tracts

			Fractional Anisotropy				Mean Diffusivity			
			AUC (p level)	Cutoff	Sensitivity	Specificity	AUC (p level)	Cutoff	Sensitivity	Specificity
Association Nuclei										
Mediodorsal	C vs	PD-N	0.57 (0.35)	>0.3847	37.25	83.33	0.49 (0.88)	<0.0009	27.45	83.33
		PD-MCI	0.50 (0.96)	<0.3466	11.11	83.33	0.56 (0.56)	>0.001	22.22	83.33
		PD-D	0.60 (0.32)	<0.3464	40.00	83.33	0.78 (<0.01)	>0.001	53.33	83.33
	PD-N vs	PD-MCI	0.57 (0.37)	<0.3542	22.22	80.39	0.53 (0.71)	>0.0011	27.78	80.39
		PD-D	0.65 (0.11)	<0.3524	40.00	80.39	0.77 (<0.01)	>0.001	60.00	80.39
	PD-MCI vs	PD-D	0.61 (0.32)	<0.3505	40.00	83.33	0.74 (<0.01)	>0.001	46.67	83.33
Lateral Posterior	C vs	PD-N	0.55 (0.49)	<0.3888	27.45	83.33	0.54 (0.59)	>0.001	15.69	83.33
		PD-MCI	0.61 (0.22)	<0.3874	38.89	83.33	0.60 (0.28)	>0.001	27.78	83.33
		PD-D	0.50 (0.98)	<0.3836	33.33	83.33	0.62 (0.20)	>0.001	20.00	83.33
	PD-N vs	PD-MCI	0.57 (0.41)	<0.3823	27.78	80.39	0.55 (0.47)	>0.01	16.67	80.39
		PD-D	0.53 (0.75)	>0.4537	33.33	80.39	0.57 (0.39)	>0.001	13.33	80.39
	PD-MCI vs	PD-D	0.59 (0.39)	>0.454	33.33	83.33	0.52 (0.89)	>0.001	13.33	83.33
Pulvinar	C vs	PD-N	0.60 (0.19)	>0.444	21.57	83.33	0.59 (0.21)	<0.0009	31.37	83.33
		PD-MCI	0.55 (0.62)	<0.3756	33.33	83.33	0.58 (0.37)	>0.0011	22.22	83.33
		PD-D	0.55 (0.63)	<0.3719	33.33	83.33	0.64 (0.11)	>0.0011	33.33	83.33
	PD-N vs	PD-MCI	0.63 (0.09)	<0.3863	38.89	80.39	0.67 (0.03)	>0.001	38.89	80.39
		PD-D	0.62 (0.16)	<0.3863	40.00	80.39	0.77(<0.01)	>0.001	60.00	80.39
	PD-MCI vs	PD-D	0.50 (0.97)	<0.3558	13.33	83.33	0.58 (0.43)	>0.0011	6.67	83.33
Limbic Nuclei										
Anterior Principal	C vs	PD-N	0.62 (0.09)	>0.3766	41.18	83.33	0.53 (0.65)	<0.0009	25.49	83.33
		PD-MCI	0.48 (0.86)	>0.3766	27.78	83.33	0.53 (0.73)	>0.0011	16.67	83.33
		PD-D	0.68 (0.04)	<0.3292	40.00	83.33	0.81 (<0.01)	>0.0011	60.00	83.33
	PD-N vs	PD-MCI	0.64 (0.05)	<0.3412	33.33	80.39	0.56 (0.40)	>0.0011	16.67	80.39
		PD-D	0.78 (<0.01)	<0.3401	53.33	80.39	0.81 (<0.01)	>0.0011	60.00	80.39
	PD-MCI vs	PD-D	0.68 (0.06)	<0.3254	40.00	83.33	0.82 (<0.01)	>0.0011	66.67	83.33
Lateral Dorsal	C vs	PD-N	0.56 (0.41)	>0.3798	25.49	83.33	0.60 (0.15)	<0.001	35.29	83.33
		PD-MCI	0.57 (0.41)	<0.297	16.67	83.33	0.56 (0.51)	>0.0013	22.22	83.33
		PD-D	0.77 (<0.01)	<0.3011	46.67	83.33	0.74 (<0.01)	>0.0013	60.00	83.33
	PD-N vs	PD-MCI	0.63 (0.08)	<0.3228	38.89	80.39	0.66 (0.03)	>0.0012	33.33	80.39
		PD-D	0.80 (<0.01)	<0.3211	80.00	80.39	0.80 (<0.01)	>0.0012	66.67	80.39
	PD-MCI vs	PD-D	0.73 (0.01)	<0.3011	46.67	83.33	0.68 (0.06)	>0.0013	53.33	83.33

Table 10-8 Continued

			Fractional Anisotropy				Mean Diffusivity			
			AUC (*p* level)	Cutoff	Sensitivity	Specificity	AUC (*p* level)	Cutoff	Sensitivity	Specificity
Sensory Nucleus										
Ventral Posterior	C vs	PD-N	0.49 (0.87)	<0.4194	9.80	83.33	0.50 (0.99)	<0.0008	15.69	83.33
		PD-MCI	0.55 (0.56)	<0.4181	16.67	83.33	0.66 (0.09)	>0.0009	50.00	83.33
		PD-D	0.49 (0.87)	<0.4194	9.80	83.33	0.79 (<0.01)	>0.0009	66.67	83.33
	PD-N	PD-MCI	0.56 (0.44)	<0.4283	22.22	80.39	0.64 (0.11)	>0.0009	44.44	80.39
		PD-D	0.52 (0.82)	<0.4296	33.33	80.39	0.79 (<0.01)	>0.0009	60.00	80.39
	PD-MCI	PD-D	0.54 (0.69)	>0.4561	46.67	83.33	0.62 (0.25)	>0.0009	26.67	83.33
Motor Nuclei										
Ventral Anterior	C vs	PD-N	0.57 (0.38)	>0.4537	19.61	83.33	0.54 (0.59)	<0.0008	13.73	83.33
		PD-MCI	0.56 (0.52)	<0.3816	22.22	83.33	0.62 (0.17)	>0.0009	27.78	83.33
		PD-D	0.64 (0.15)	<0.3818	40.00	83.33	0.84 (<0.01)	>0.0009	60.00	83.33
	PD-N	PD-MCI	0.63 (0.13)	<0.4047	38.89	80.39	0.67 (0.03)	>0.0009	44.44	80.39
		PD-D	0.73 (<0.01)	<0.4047	53.33	80.39	0.87 (<0.01)	>0.0009	86.67	80.39
	PD-MCI	PD-D	0.60 (0.34)	<0.3814	33.33	83.33	0.70 (0.04)	>0.001	33.33	83.33
Ventral Lateral	C vs	PD-N	0.50 (0.97)	<0.41	15.69	83.33	0.52 (0.77)	>0.0009	21.57	83.33
		PD-MCI	0.57 (0.44)	<0.4079	22.22	83.33	0.62 (0.18)	>0.0009	33.33	83.33
		PD-D	0.51 (0.91)	<0.4099	20.00	83.33	0.78 (<0.01)	>0.0009	53.33	83.33
	PD-N	PD-MCI	0.55 (0.54)	<0.4165	33.33	80.39	0.61 (0.14)	>0.0009	33.33	80.39
		PD-D	0.51 (0.92)	>0.4534	26.67	80.39	0.78 (<0.01)	>0.0009	53.33	80.39
	PD-MCI	PD-D	0.57 (0.53)	>0.453	26.67	83.33	0.66 (0.11)	>0.0009	20.00	83.33
Non-Specific Nucleus										
Centromedian/ Parafascicular	C vs	PD-N	0.50 (0.99)	<0.3685	23.53	83.33	0.50 (0.99)	>0.0009	11.76	83.33
		PD-MCI	0.57 (0.45)	<0.3694	33.33	83.33	0.56 (0.51)	>0.0009	27.78	83.33
		PD-D	0.49 (0.93)	>0.4419	13.33	83.33	0.76 (<0.01)	>0.0009	53.33	83.33
	PD-N	PD-MCI	0.55 (0.57)	<0.3579	22.22	80.39	0.56 (0.45)	>0.0009	33.33	80.39
		PD-D	0.50 (0.96)	<0.3564	13.33	80.39	0.75 (<0.01)	>0.0009	60.00	80.39
	PD-MCI	PD-D	0.57 (0.52)	>0.4189	33.33	83.33	0.70 (0.03)	>0.001	46.67	83.33

AUC: area under the receiver operating curve (chance = 0.5; perfect separation = 1.0)

9.6.5 The combined contribution of nuclei and nuclei tract integrity in cognitive dysfunction

To further aid in discrimination of the cognitively impaired PD patients from the unimpaired PD patients and healthy control subjects the diffusion parameters of both the thalamic nuclei and the associated nuclei tracts were analysed together. We have previously established the contribution that each thalamic nucleus makes to cognitive domain (*Table 8-6*) and here have determined that the tracts arising from these nuclei make little contribution to cognition. This final analysis examines the combined effect of thalamic nuclei and their associated tracts over cognitive dysfunction. In order to determine the combined effect of nuclei and their tracts on cognitive dysfunction we first had to determine the association between the thalamic nuclei and their tracts. Results are presented in *Table 9-9.*

9.6.5.1 Fractional ainsotropy

We first conducted a series of Pearson correlations to determine the association between each nuclei tract and the nuclei themselves. Results (light grey) were as expected, each thalamic nuclei tract showed an association with the nuclei it originated from. For each tract, the strongest correlation was with the nucleus it was seeded from (white). A series of multiple regression analyses were then conducted for each tract. All nuclei that had an association with that tract were entered to determine if the nuclei was an independent predictor of its associated tract, or if there were underlying effects of surrounding nuclei contributing to this association. Multiple regression results (dark grey) were mostly as expected. For the association MDn, LP and Pu, the limbic LD, sensory VP, motor VL and non-specific CM/Pf nuclei tracts the strongest independent predictor of FA values of that tract was the FA values of the associated nucleus. In some cases, other surrounding thalamic nuclei also strongly contributed to a nuclei tract other than the one seeded from it. In the case of the MDn and LP tracts the Pu was also a unique predictor, in the case of the VP tract the VL nucleus was an additional unique predictor and in the case of the CM/Pf tract the LP nucleus was a unique predictor, suggesting a significant amount of shared variance between these nuclei. For the limbic AP, and motor VA tracts, nuclei other than the one where the tract originated from were the strongest unique predictors of FA tract values. For the AP tract the other limbic LD nuclei was the strongest unique predictor and

for the VA the other motor VL nucleus was the strongest predictor, indicating that these nuclei have shared connectivity arising from their functional roles.

9.6.5.2 Mean diffusivity

With exception to the motor VA and VL nuclei, in terms of correlation analysis each nuclei showed the strongest relationship with its associated tract when measured with MD. The VA tract had stronger associations with the MDn and VP nuclei while the VL tract had stronger associations with the LD, VP, VA and CM/Pf nuclei. When entered into the multiple regression model results were as expected, every tract was uniquely predicted by its associated nucleus. There were no cases where a stronger unique predictor was found. There were two instances where there was an additional nucleus which uniquely predicted some of the variance in MD values of tracts however. The first was the VA tract where the MDn was also a unique predictor and the second was the CM/Pf where the VA was also a unique predictor.

Table 9-9: Association between thalamic nuclei and corresponding thalamic nuclei tracts

Nuclei tracts	Statistic	MDn	LP	Pu	AP	LD	VP	VA	VL	CM/Pf
Fractional Anisotropy										
Association Nuclei										
MDn	*r*	0.36***	0.10	0.07	0.19	0.16	0.20*	0.20*	0.16	0.23*
	β	0.23*					0.17	-0.01		0.12
LP	*r*	0.34***	0.35***	0.21*	0.22*	0.10	0.10	0.18	0.07	0.26**
	β	0.19	0.21*		0.10					0.18*
Pu	*r*	0.35***	0.33†	0.26***	0.27**	0.17	0.07	0.11	0.10	0.09
	β	0.20*	0.21*	0.20*	0.13					
Limbic Nuclei										
AP	*r*	0.22*	0.00	0.21*	0.39***	0.24*	0.10	0.22*	0.11	0.17
	β	0.02			0.25**	0.10		0.11		
LD	*r*	0.09	-0.02	0.13	0.39***	0.63***	0.04	0.16	0.14	0.12
	β				0.29***	0.61***				
Sensory Nucleus										
VP	*r*	0.10	0.23*	0.21*	-0.01	0.10	0.40***	0.24*	0.23*	0.11
	β		0.14				0.34***	0.11	0.13	
Motor nuclei										
VA	*r*	0.11	0.11	-0.02	0.03	0.03	0.19	0.36***	0.20*	0.11
	β							0.14	0.05	
VL	*r*	0.18	0.24*	0.06	0.13	0.10	0.32**	0.44***	0.43***	0.12
	β		0.16				0.23*	0.38***	0.38***	
Non-specific nucleus										
CM/Pf	*r*	0.34***	0.11	-0.03	0.30**	0.14	0.07	0.24*	0.13	0.34***
	β	0.18			0.14			0.20*		0.26**

Pearson correlations (*r*) and Beta values (β) of the association between thalamic nuclei (column 1) and the tracts that are seeded from them (column 2). All participants were included. Significant associations are denoted: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$, Beta values are only reported for those comparisons that have a significant correlation. **White:** relationship between nucleus and its associated tract; **Light grey:** correlation results; **dark grey:** multiple regression results.

Table 9-9 Continued

Nuclei tracts	Statistic	MDn	LP	Pu	AP	LD	VP	VA	VL	CM/Pf
Mean Diffusivity										
Association Nuclei										
MDn	*r*	0.51†	0.22*	0.24*	0.35†	0.18	0.30**	0.53†	0.24*	0.27**
	β	0.34**	-0.02	0.01	0.09		0.01	0.23*	-0.04	0.03
LP	*r*	0.21*	0.33†	0.17	0.11	0.03	0.17	0.30**	0.12	0.15
	β	-0.05	0.20		-0.17			0.00		-
Pu	*r*	0.26**	0.09	0.39†	0.21	0.15	0.23	0.36†	0.16	0.18
	β	0.06		0.29**	0.06			0.10		
Limbic Nuclei										
AP	*r*	0.38†	0.27**	0.26**	0.47†	0.29**	0.30†	0.43†	0.24*	0.20*
	β	0.16	0.15	0.12	0.40†	0.13	0.09	0.15	0.07	0.07
LD	*r*	0.15	0.18	0.23*	0.21*	0.69†	0.22*	0.27**	0.26**	0.16
	β			0.15	0.09	0.66†	0.17	0.15	0.22*	
Sensory Nucleus										
VP	*r*	0.43†	0.25**	0.27**	0.31**	0.11	0.44†	0.48†	0.35†	0.31†
	β	0.17	-0.04	0.04	0.03		0.35**	0.12	0.22	0.10
Motor nuclei										
VA	*r*	0.31**	0.30	0.24†	0.24*	-0.01	0.32†	0.47†	0.26†	0.26**
	β	-0.07	0.11	0.08	0.04		0.16	0.27*	0.09	0.17
VL	*r*	0.32**	0.29**	0.19*	0.23*	0.07	0.25**	0.36†	0.25**	0.23*
	β	0.10	0.10	-0.01	0.02		-0.14	-0.11	-0.01	-0.09
Non-specific nuclei										
CM/Pf	*r*	0.25**	0.21*	0.18	0.27**	0.21*	0.25*	0.31**	0.26**	0.36†
	β	-0.01	0.08		0.17	0.14	0.06	0.07	0.13	0.29**

Pearson correlations (*r*) and Beta values (β) of the association between thalamic nuclei (column 1) and the tracts that are seeded from them (column 2). All participants were included. Significant associations are denoted: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$, Beta values are only reported for those comparisons that have a significant correlation. **White:** relationship between nucleus and its associated tract; **Light grey:** correlation results; **dark grey:** multiple regression results.

Once it was determined that tractography values are mostly uniquely predicted by their associated nuclei, we examined the influence the combination of each of these has on cognition by performing a series of backward stepwise linear regressions in a manner similar to that described previously in this thesis (*Section 8.7.5.2*). Briefly, all nuclei that showed an association with a cognitive domain were entered into a backward stepwise regression to firstly ascertain the total amount of variance accounted for in that cognitive domain (R^2 for model) and also to determine the contribution each nucleus made to this (R^2 change). Following this, a second model was created whereby all associated thalamic nuclei tracts were entered to determine if this increased the variance accounted for in each cognitive domain, and the individual contribution each thalamic nuclei tract made to this. Ten models were created, two for each cognitive domain. Only MD measures of thalamic nuclei and tracts were used in this instance as they showed a stronger association with cognition than FA measures. Results are presented in *Table 9-10.*

For each nucleus, adding the MD measures of its associated nuclei tract increased the variance accounted for in cognition. This increase was only significant for the association and limbic thalamic nuclei however, and mainly in the domains of attention and executive function. For the attention domain adding the nuclei tract arising from the Pu increased the variance accounted for in attention scores. For the executive function domain adding the MDn, Pu and AP tracts gave a significant increase. For the visuospatial/visuoperception domain adding thalamic nuclei tracts did not increase the overall proportion of variance accounted for, nor the variance accounted for by each individual nuclei. The same is true for the learning and memory domain. In the global domain, the MDn is again implicated, with a significant increase in the proportion of variance accounted for in scores once the nuclei tract is added.

Table 9-10: The variance in cognition accounted for by the combined MD measures of thalamic nuclei and tracts

	Model 1 Nuclei associated with cognition				Model 2 Adds tracts of those nuclei				Model 1 vs Model 2	
	Beta	p	R^2	p	Beta	p	R^2	p	R^2 change	p
Attention										
Models	**R^2 = 32%, F(5,78) = 7.31, p<0.01**				**R^2 = 34%, F(10,73) = 3.82, p<0.01**				2%	0.75
MDn	-0.27	0.03	12%	<0.01	-0.17	0.16	14%	<0.01	2%	0.16
Pu	-0.21	0.07	11%	<0.01	-0.27	0.02	17	<0.01	6%	0.02
LD	-0.22	0.13	15%	<0.01	-0.24	0.10	17%	<0.01	3%	0.10
VP	-0.22	0.07	10%	<0.01	-0.21	0.08	14%	<0.01	3%	0.08
CM/Pf	-0.28	0.02	10%	<0.01	-0.11	0.36	11%	<0.01	1%	0.36
Executive Function										
Models	**R^2 = 27%, F(7,76) = 3.93, p<0.01**				**R^2 = 34%, F(14,69) = 2.51, p<0.01**				0.7%	0.40
MDn	-0.33	<0.01	14%	<0.01	-0.11	0.38	0.8%	0.38	15%	<0.01
LP	-0.22	0.07	5%	=0.10	-0.04	0.74	5%	0.10	0.01%	0.10
Pu	-0.18	0.12	9%	<0.01	-0.25	0.03	14%	0.01	5%	0.03
AP	-0.13	0.28	6%	0.03	-0.25	0.04	10%	0.01	5%	0.04
LD	-0.10	0.51	8%	<0.01	-0.27	0.07	12%	<0.01	4%	0.07
VP	-0.28	0.02	13%	<0.01	-0.17	0.16	15%	<0.01	2%	0.16
CM/Pf	-0.30	<0.01	10%	<0.01	-0.04	0.74	10%	0.01	0.01%	0.74
Visuospatial/Visuoperception										
Models	**R^2 = 35%, F(7,76) = 5.80, p<0.01**				**R^2 = 38%, F(14,69) = 3.05, p<0.01**				3%	0.80
MDn	-0.32	<0.01	15%	<0.01	-0.14	0.25	16%	<0.01	1%	0.25
LP	-0.31	<0.01	10%	<0.01	-0.03	0.79	10%	0.02	0.01%	0.79
Pu	-0.22	0.07	8%	<0.01	-0.14	0.25	10%	0.02	0.02%	0.25
AP	-0.16	0.20	5%	0.04	-0.14	0.25	7%	0.06	2%	0.25
LD	-0.33	0.02	15%	<0.01	-0.33	0.02	15%	<0.01	0.01%	0.57
VP	-0.33	<0.01	14%	<0.01	-0.08	0.51	14%	<0.01	0.01%	0.51
CM/Pf	-0.36	<0.01	13%	<0.01	0.01	0.90	13%	<0.01	0.01%	0.90
Learning and Memory										
Models	**R^2 = 30%, F(7,76) = 4.68, p<0.01**				**R^2 = 33%, F(14,69) = 2.40, p = 0.01**				3%	0.91
MDn	-0.40	<0.01	20%	<0.01	-0.08	<0.01	20%	<0.01	0.01%	0.51
LP	-0.23	0.05	5%	0.04	0.02	0.89	5%	0.13	0.01%	0.88
Pu	-0.20	0.09	8%	<0.01	-0.18	0.14	10%	0.01	2%	0.14
AP	-0.19	0.13	5%	0.03	-0.19	0.13	8%	0.03	3%	0.13
LD	-0.25	0.09	9%	<0.01	-0.06	0.69	9%	0.02	0.01%	0.69
VP	-0.34	<0.01	15%	<0.01	-0.09	0.43	16%	<0.01	1%	0.43
CM/Pf	-0.28	0.02	10%	<0.01	-0.80	0.49	10%	0.01	0.01%	0.49
Global Z Score										
Models	**R^2 = 38%, F(7,76) = 6.53, p<0.01**				**R^2 = 42%, F(14,69) = 3.56, p<0.01**				4%	0.64
MDn	-0.36	<0.01	19%	<0.01	-0.15	0.20	21%	<0.01	2%	0.01
LP	-0.23	0.05	6%	0.02	-0.03	0.79	6%	0.08	0.01%	0.79
Pu	-0.23	0.05	11%	<0.01	-0.23	0.05	16%	<0.01	5%	0.05
AP	-0.15	0.22	7%	0.02	-0.24	0.04	11%	<0.01	5%	0.04
LD	-0.24	0.10	14%	<0.01	-0.19	0.18	16%	<0.01	2%	0.18
VP	-0.33	<0.01	16%	<0.01	-0.15	0.20	18%	<0.01	2%	0.20
CM/Pf	-0.35	<0.01	13%	<0.01	-0.05	0.64	14%	<0.01	0.2%	0.64

Model 1 shows the combined contribution of thalamic nuclei to each cognitive domain. Model 2 shows the increased contribution gained after adding associated nuclei tracts. In most instances adding tracts does not increase the proportion of variance that is accounted for in cognitive scores by a significant amount. Beta values for each nucleus and tract are also provided to give an indication of each individual contribution to cognition and reflect the values given in the individual models of nuclei and tracts, not in the overall model – significant beta values for the overall model have previously been reported (*Table 9-7*). The thalamic nuclei independently influence cognition more than the nuclei tracts do. Only PD patients have been included in analysis.

As expected, including covariates in Model 2 increased the proportion of variance accounted for in each cognitive domain. In the attention domain R^2 increased to 45% [$F(14,69) = 4.08$, $p < 0.01$] and none of the nuclei or tracts were independent predictors. In the executive function domain R^2 increased to 52% [$F(18,65) = 3.89$, $p < 0.01$] and again no nuclei or tracts were independent predictors of executive function. In the visuospatial domain however the LD nucleus remained an independent predictor of domain score after the inclusion of covariates. R^2 increased to 44% in this model [$F(18,65) = 2.89$, $p < 0.01$]. In the learning and memory domain R^2 increased to 39%, [$F(18,65) = 2.35$, $p < 0.01$] and there were no regions which independently predicted this cognitive domain after the inclusion of covariates. Finally, in the overall measure of global cognition R^2 increased to 53% after the inclusion of covariates [$F(18,65) = 4.04$, $p < 0.001$] but no regions were independent predictors.

9.6.6 Using combined thalamic nuclei and tracts to aid in group discrimination

Logistic regression analyses were applied to determine the degree to which groups could be separated when taking into account both diffusion parameters of thalamic nuclei and thalamic nuclei tracts. For each pairwise comparison Beta values for each measure (FA and MD) of each thalamic nuclei and each thalamic nuclei tract are reported in *Appendix B: Logistic regression results*. The total model was tested for significance using Wald's chi-square. The success rates for identification of cognitive decline within the patient group and for identification of PD from the healthy control group are reported in *Figure 9-3.*

9.6.6.1 Identification of the patient group

The best nuclei and tracts to predict PD-N from healthy normal controls was the Pu. Within this model the best independent predictor of PD-N was MD measures of the Pu nuclei followed by FA measures of the Pu tract.

9.6.6.2 Identification of dementia

Successful identification of the PD-D group from the healthy control group was achieved with high success (71.79 - 87.18) and from the PD-N group to a similar degree (74.24 – 86.36). The best region to aid in discrimination of the PD-D group from the healthy controls was again the Pu, with FA measures of the Pu tract the strongest independent predictor, and MD measures of the Pu nucleus a close second. Surprisingly, identifying

PD-D from the PD-N sample was best achieved by the VA motor nucleus where the best independent predictor was the FA measure of the VA tract, followed by the MD measure of the VA nucleus. Separation of PD-D and PD-MCI was again one of the most difficult pairwise comparisons where only the AP, CM/Pf and VA regions had an overall model which was significant. Of these three regions the AP region showed the highest level of correct identifications. Within this model MD of the AP thalamic nuclei tract was a very slightly better independent predictor of dementia than FA of the AP thalamic nuclei tract.

9.6.6.3 Identification of mild cognitive impairment

Identification of PD-MCI was achieved 50% - 74.43% of the time from the healthy control group with higher accuracy (71.01% - 76.81%) than was possible when separating PD-MCI from the PD-N group. The Pu nucleus was again implicated, giving the highest degree of success rate in both comparisons. Within the models MD of the Pu was the strongest independent predictor followed by FA of the Pu tract when using the healthy control group as a comparison with the reverse true for the comparison with the PD-N group (FA of the Pu tract was the strongest predictor followed by MD of the Pu nucleus).

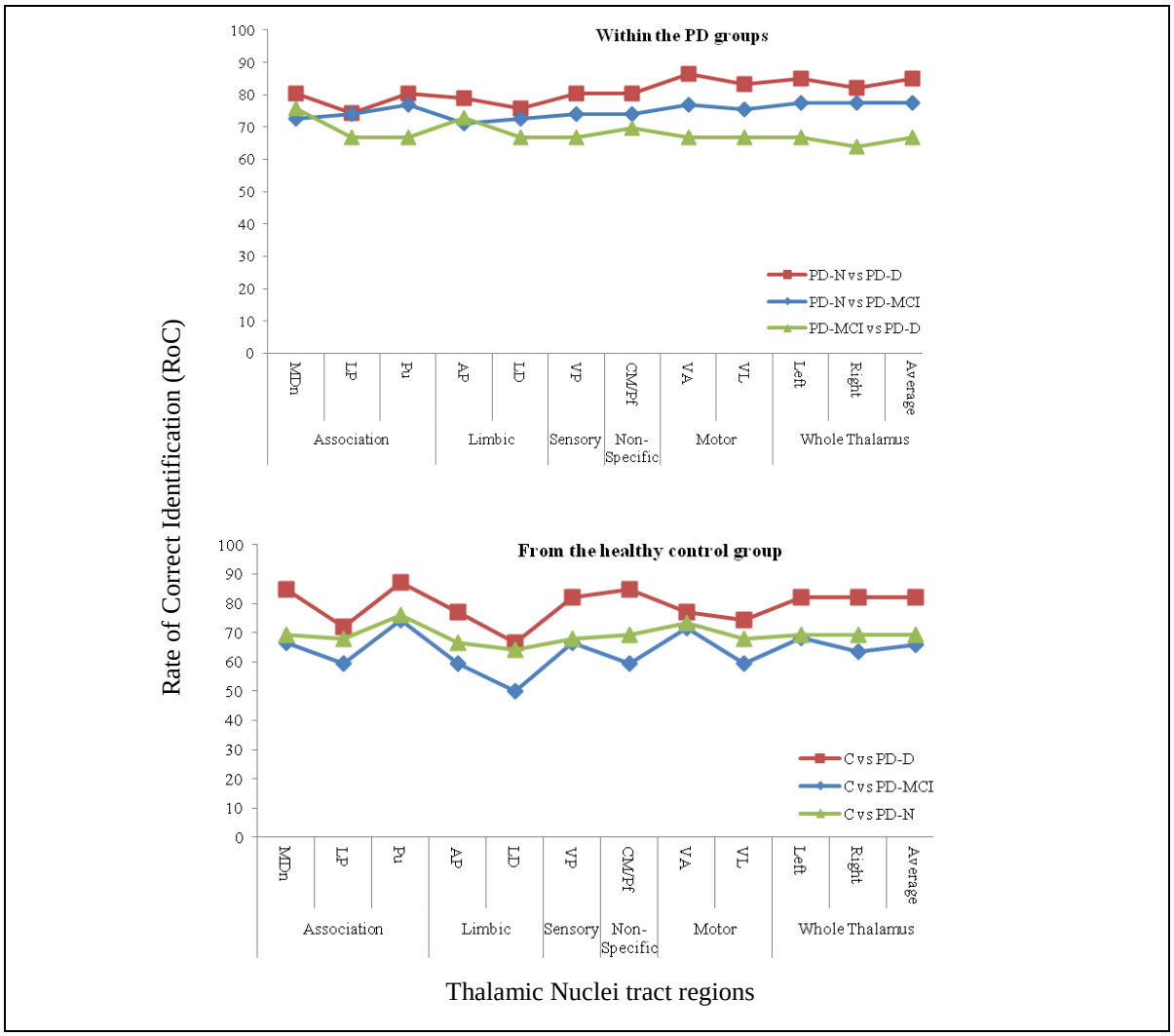


Figure 9-3: Identification of cognitive declining Parkinson's disease groups
Separation of the PD groups is possible using diffusion parameters of the thalamus and associated tracts (top). The success rate increases when analysis is restricted to individual thalamic regions, with the association and limbic nuclei regions particularly useful here. Separation of PD from normal healthy controls is also able to be achieved with some success (bottom).

9.7 Discussion

9.7.1 Summary of the results

The fibre network in Parkinson's disease is significantly disrupted, with the anterior tracts arising from the limbic thalamic nuclei most affected. Degeneration within the fibre network is generally only identifiable in the group with Parkinson's disease dementia and not evident in the groups exhibiting milder forms of cognitive dysfunction. Although others have previously examined individual thalamic tracts in neurodegenerative or psychological disease we are the first to isolate all fibre tracts originating from the thalamus and classify them by region. Very few have quantitatively examined the influence of thalamic fibre tracts on dysfunction, although some have determined degeneration of the cortico-spinal tract contributes to motor dysfunction in PD. We have gone one step further and examined the influence of the thalamic nuclei and each of the thalamic nuclei tracts in conjunction with each other, and independently to determine their association with cognitive dysfunction in Parkinson's disease. Only FA measures of the LD tract and MD measures of the VA tract had an independent influence on cognition. The LD was an independent predictor of attention, executive function and visuospatial cognitive domain scores and the VA an independent predictor of the attention and visuospatial cognitive domain scores. The influence of the fibre tracts was not as great as the influence of the thalamic nuclei and alone, diffusion measures of the fibre tracts did not adequately discriminate between the different cognitive subtypes of PD. In conjunction with the thalamic nuclei however successful identification of PD-D and PD-MCI was possible at higher levels than possible using diffusion measures of the thalamic nuclei only.

9.7.2 Tract disruption

The integrity of the whole thalamus tracts did show significant disruption in relation to the levels of cognitive dysfunction. Reflective of mean diffusivity results in the whole thalamus (*Section 6.7.4*), there was a significant increase in the PD-D group relative to all other groups. These results confirm previous reports from our own group (Melzer, et al., 2011a). The anterior thalamic radiation, of which the tracts arising from the whole thalamus can be assumed to join, shows significant disruption in PD. In contrast to our results which demonstrated increases in PD-D relative to both control subjects and to PD-N, increased diffusivity was evident in all PD (PD-D; PD-MCI; PD-N) groups relative to

controls in the Melzer, et al., (2011a) sample but not in PD-D or PD-MCI relative to PD-N. We have perhaps therefore demonstrated here that integrity disruption in PD-D is an artifact of dementia rather than motor symptoms in PD for the first time.

The tracts arising from the individual thalamic nuclei are more sensitive to cognitive dysfunction than the whole thalamus tracts. Alongside the MDn, the AP tract is the only other tract to show a significant increase in diffusivity in PD-D relative to PD-MCI and the LD the only tract to show a significant increase in PD-MCI relative to PD-N. The LD nucleus is involved in the episodic memory pathway (Aggleton & Brown, 1999; Cipolotti, et al., 2008) and also mediates executive function (van der Werf, 2003) so fiber disruption here could be reflective of the two earliest symptoms of cognitive dysfunction in PD (Janvin, et al., 2006). The nuclei involved in this pathway (both AP and LD) appear to be relatively spared by LB pathology in PD (Halliday, 2009), suggesting the communication breakdown between these subcortical regions and the cortex is an artifact of network disruption. Results from our previous chapter support this idea, the AP is the only region to show no mean diffusivity changes in any of the PD groups (*Section 8.7.4*), although the fiber tract shows significant MD changes in PD-D relative to all other groups.

Degeneration of cortical tracts is thought to be due to the loss of cells in connecting regions (Hulshoff Pol, et al., 2000) and, as the anterior thalamic radiation is one of the major fiber tracts connecting the thalamus to the cortex it could reflect degeneration in multiple other areas of the thalamus which also project to the frontal lobes and mediate frontal symptoms such as the MDn (Kemether, et al., 2003). Indeed, the tract specifically delineated from the MDn shows significant disruption in PD-D relative to all other groups in this sample.

The influence of the anterior thalamic radiation, or the tract arising from the AP in this case has to be considered carefully in our sample as there was a significant group x hemisphere interaction here, even after controlling for co-variates. Follow up analyses indicated a significant group effect in the left tract, but not the right. Previous chapters also report that the left whole thalamus is larger and has higher FA than the right. In regards to the thalamic nuclei, the MDn was larger and had higher FA in the left, the LD was larger in the left hemisphere, the AP, VA, VP and Pu had higher FA in the left and the VP had lower MD in the left hemisphere. The greater integrity in the left doesn't appear to have unduly influenced results however. The right hemisphere can be loosely described as influencing non-verbal material whilst the left mediates verbal tasks but the cognitive

profile of our cohort includes deficits of both verbal and visual memory. Cipolotti, (2008) is one of the few studies to have previously examined the lateralisation effect in regards to cognition in PD and reports that the left hemisphere was more affected than the right in the patients, a result that is in direct contrast to ours. Although Cipolotti only examined the mammillothalamic tract in PD patients, FA reduction was localised to the left tract. Despite this, the cognitive profile of the patients was similar to our own as they exhibited equal involvement of verbal and visual dysfunction. Given the myriad of connectivity between the left and right thalamus it is not unreasonable to assume that although our results indicate degeneration appears to be worse in the right hemisphere, connectivity disruption between the left and right thalamus dictates that this will manifest as both verbal and visual dysfunction. The few previous studies that have reported differential hemisphere influence on cognition in regards to the thalamus support this idea. Peran (2009) showed significant cellular disruption bilaterally which aided in discrimination between PD and controls and although Summerfield (2005) reports that only the left thalamus was degenerated in PD, this had a significant association with the presence of dementia.

Across the spectrum of PD in this sample the tracts of the motor nuclei (VA, VL) are also significantly disrupted in PD-D relative to PD-N (VL) and the VA is the only tract to show progressive disruption from PD-N to PD-MCI through to PD-D. These results confirm a previous study (Gupta, 2012) of the motor pathway in PD which showed significant degeneration of the nigrostriatal and mesolimbic tracts – reflected in both decreased FA and increased MD in PD relative to healthy controls. The authors did not discriminate between the level of cognitive impairment in their sample but suggest that diffusion disruptions reflect loss of dopaminergic neurons in the early stages of the disease and that this could be used as an early biomarker. The influence of our VA tract on cognition is most likely a result of the fact that the mean diffusivity measure of the VA tract is strongly associated with the MDn nucleus. As the VA and the MDn both project from the thalamus to the frontal region (Mori, Oishi, & Faria, 2009) it is possible the tracts of these nuclei merged into the anterior thalamic tract and reflect the function of this white matter pathway, rather than the differential function of the motor or limbic pathway as intended.

9.7.3 The relationship with cognition

Similar to the results of the previous chapter there was a significant association between fiber tracts and the function of the region they terminated in. Frontal lobe dysfunction such as attention and executive function for example, was significantly associated with the limbic nuclei which project to this region. The frontal fiber network has previously been implicated in regards to dysfunction in the working memory and executive function domains in Schizophrenia (Mamah, et al., 2010). Although fractional anisotropy, not mean diffusivity was the integrity measure of choice there was significant cellular disruption in Schizophrenia patients compared to control subjects which correlated with these measures of cognition.

The influence of cortical connectivity on mild cognitive dysfunction has previously only been examined by ourselves (Melzer, et al., 2011a) and in Alzheimer's disease (Mielke, et al., 2009). Tractography was not applied in the Alzheimer's disease sample, instead regions of interest within major fiber tracts were examined. Results showed that in anterior regions; the anterior cingulum bundle and the fornix AD patients had significantly lower FA compared to control subjects. Compared to MCI patients, these regions along with the selenium were also significantly disrupted and had a significant association with a range of cognitive scores. Alongside our current results which also show significant cellular disruptions in some white matter tracts between PD-MCI and PD-D this could indicate that network connectivity is one of the major changes that contribute to the development of dementia in neurodegenerative diseases. Indeed, we have also shown that tracts implicated between PD-MCI and PD-D in our sample (MDn, AP) influence the areas of cognition most involved in the progression to dementia, the frontal and temporal regions (Beyer & Aarsland, 2008).

The progression to dementia is the likely result of multifaceted, rather than single region disruption. This thesis also extensively examined the combined and independent influence of the nuclei and associated tracts and found that in conjunction, the nuclei and their connections heavily influence cognitive dysfunction in PD but the tracts alone do not provide much added influence. The thalamic nuclei and connections have not previously been examined but, rather than influencing cognition themselves could lead to degeneration in connected areas rather than directly influencing cognition. In temporal lobe epilepsy for example (Wang, et al., 2010) patients exhibited decreased FA and

increased MD in bilateral thalamus and the white matter of connected frontal and occipital regions. DT changes were associated with impairment of tasks of executive function.

The idea of relative axonal integrity in PD is further supported by Braak (2004) who suggests that nerve cells with long robust axons that are insulated by myelin are actually protected against LN and LB formation throughout the entire course of PD. The principles of diffusion tensor imaging in relation to tractography depend on the direction of diffusion, as dictated by the integrity of the white matter axons (Watts, Liston, Niogi, & Ulug, 2003). As our FA results barley change across PD groups and the limbic tracts are the only ones to show significant degeneration, in PD-D relative to: PD-N in the case of the AP; and to PD-MCI, PD-N and control groups in the case of the LD this could be why. Furthermore, despite being one of the main influences on the DTI signal, myelin integrity does not appear to influence DTI results. Assaf & Pasternak (2008) address this directly in a review of white matter mapping and agree with this hypothesis. Experiments on myelinated and non myelinated axons also show that anisotropy values are similar (Beaulieu & Allen, 1994). The most compelling evidence comes from a multiple sclerosis sample which showed limited FA disruption in areas of white matter where demyelination is known to occur (Assaf, et al., 2002; Bammer, et al., 2000; Filippi, Cercignani, Inglese, Horsfield, & Comi, 2001). The authors of the review conclude that myelin is only partially reflected in DTI measures of FA. If the sheaths are protected against Lewy formation there is no reason for the cells to show degeneration. The discrepancy between FA and MD results is easily explained by the fact that MD is a measure of cellular integrity and more reflective of degeneration within the axon rather than the overall degeneration of direction. FA is more reflective of increases in cellular space and DTI studies in stroke patients (Sotak, 2002) show a marked reduction of FA at the lesion site. Lesion sites typically show neuronal death (and an increase in extracellular space).

The protection against LB pathology could also explain the low association between fiber tracts and cognition in this sample. Although the influence of the thalamic tracts cannot be ignored as they do add to the model of cognition, this could just be due to their association with the main area of the cortex they are connected with. It is not surprising, for example that the tracts originating from the anterior nuclei and the mediodorsal nucleus have an association with executive function and memory. These regions preferentially connect to the frontal lobes by way of the anterior thalamic radiation and disruption in this tract could just reflect long term cellular degeneration of the grey

matter structures of the nuclei. A theory heavily supported by the fact the tracts do not show degeneration until cognitive dysfunction is well advanced in PD-D in our sample.

9.7.4 Justification of the study methodology

Although very few groups have completed tractography from the thalamus, our tractography results appear to converge with those that have. The anterior thalamic radiation has been specifically targeted for examination in two cases (Lo, et al., 2011; Mahon, et al., 2009) for example and shows connectivity between the frontal lobe and thalamus, completing the fronto-striato-thalamic circuit by penetrating the thalamus and proceeding to other cortical regions, the brainstem and cerebellum. In our sample we used the anterior principal nucleus as a seed point and created a strong fibre tract which projected from here to the prefrontal cortex and which can be assumed to represent the anterior thalamic tract as this tract is associated with anterior and mediodorsal thalamic regions (Wanaka, et al., 2004).

The mediodorsal and centromedian/parafascicular thalamic nuclei are the only other two regions to be targeted for tractography investigation (Eckert, et al., 2012). Results in healthy subjects confirm the pattern of connectivity we have shown in our patient sample. The MDn connected with the dorsolateral prefrontal cortex, and regions of the anterior cingulate cortex. The CM/Pf showed a different pattern, connecting with the frontal cortex and not continuing into prefrontal regions. This study also allowed for examination of subcortical connections and results converge with previous functional and animal studies. The MDn forms part of the relay system between the basal ganglia and cortex; part of the dorsolateral-prefrontal loop between the caudate nucleus and the cortex; and part of the limbic network between the cingulate cortex and MDn, systems fundamental to cognitive, motivation and movement processing (Haber & Mcfarland, 2001). The CM/Pf had strong connectivity with the pallidum, putamen, hippocampus and amygdala. We did not allow for examination of the degree of connectivity between structures as Eckert, (2012) did so are restricted to visual examination of connectivity only. This does not lead to easy identification of connectivity between thalamic regions and surrounding subcortical structures but our CM/Pf tracts do appear to agree with those of Eckert and colleagues, passing through the region of the basal ganglia and limbic system, whilst the MDn bypasses the basal ganglia, instead projecting through the cingulate to the cortex.

Unfortunately DTI does not allow for anterograde and retrograde connections to be differentiated (Leh, et al., 2007). We cannot, therefore establish whether the fibre tracts are travelling to or from the thalamic nuclei. Despite this limitation, DTI still offers a valuable contribution in understanding the organisation of thalamic network connectivity, and the disruption which is evident in PD.

9.7.5 Summary

Our tractography study provides, for the first time, an evaluation of all major fiber pathways between the thalamus and the cortex. We have shown there is a significant disruption in network connectivity in PD-D, and that this is mainly confined to those tracts originating from anterior thalamic regions, assumed to represent the anterior thalamic radiation. In conjunction with the integrity of the thalamic nuclei it is possible to use measures of integrity of tracts to discriminate between those PD patients with no cognitive impairment, and those with mild cognitive impairment. The integrity of the fiber tracts alone does not allow for much discrimination between groups however as, generally degeneration is only evident in the PD-D group and not prior to this in the PD-MCI group. Although few studies have applied tractography to investigate the connectivity of the thalamus (Leh, et al., 2008; Lo, et al., 2011; Mahon, et al., 2009) our findings are in accordance with anatomical guidelines of white matter fiber tract based atlases (Wanaka, et al., 2004) and agree with the two studies that have delineated tracts from the AP, MD and CM/Pf thalamic nuclei (Eckert, et al., 2012; Jakab, et al., 2012). Examination of network integrity in Parkinson's disease alongside examination of cellular integrity in the thalamus could allow for detailed examination of disease progression and efficacy of interventions longitudinally.

Chapter 10. SUMMARY, LIMITATIONS AND RECOMMENDATIONS

10.1 Summary

This thesis applied multiple imaging techniques in a large sample of Parkinson's disease patients stratified by stage of cognitive decline. We have shown that thalamic degeneration is a significant factor in the cognitive symptoms of this disorder. Traditional structural based imaging methods showed significant levels of whole thalamic atrophy in Parkinson's disease with dementia and a significant relationship with multiple facets of cognition across the whole sample. This component of the thesis also examined the microstructural integrity of the whole thalamus and showed cellular disruption, as evidenced by increased mean diffusivity in the thalamus in Parkinson's disease with mild cognitive impairment. Mean diffusivity measures of integrity were more sensitive to cognition and, overall had a stronger relationship within multiple cognitive domains. In contrast to previous reports (Peran, et al., 2009) this study did not identify any relationship between fractional anisotropy measures of thalamic integrity and Parkinson's disease.

In an effort to compare two commonly applied methodologies in the same sample, the whole thalamus was also examined using a region of interest approach in standard space. In contrast to other studies that have used a whole brain approach (Burton, et al., 2005; Summerfield, et al., 2005) our standard space results did not show any regions of thalamic reduction in any of the Parkinson's disease groups. This result does confirm a previous study generated by our own group however who examined the whole brain in a similar sample of PD patients and report no areas of thalamic change (Melzer, et al., 2011b). The relationship between mean diffusivity measures of the thalamus and cognition remained in standard space with the greatest changes concentrated to ventral and centromedian/parafascicular regions. These results supported what has previously been identified *in vivo* in one Parkinson's sample (McKeown, et al., 2008) and what has previously been identified from histology studies at autopsy (Halliday, 2009; Henderson, et al., 2000a).

For the first time in a neurodegenerative sample, we applied a *k* means clustering technique (Behrens, et al., 2003) to the diffusion tensor images and segmented the thalamus into several clusters where each cluster was assumed to represent the major subdivisions of the thalamus. These thalamic nuclei showed different levels of cellular, and volumetric disruption according to their anatomical location. As in the whole

thalamus, mean diffusivity measures were more sensitive to cognitive dysfunction, showing significant disruption in some areas in the PD-N group, worsening and affecting further regions in the PD-MCI group and eventually showing disruption at a higher level in all nuclei in PD-D. Most regions had a significant association with expected domains of cognition, although there were some unexpected findings which suggested the nuclei work in unison to moderate behaviour rather than operating as independent units, an idea supported by the extensive research that has been conducted by Braak (2008).

This thesis also identified the major fiber pathways that originated from each thalamic nucleus to explore the differential involvement of nuclei in Parkinson's disease. This is the first time this has been conduced in PD using all of the thalamic nuclei as seed points, although our group has previously applied a whole brain tractography approach and shown that the anterior tracts are most affected in groups with worse cognitive dysfunction in PD (Melzer, et al., 2011a). We found that the fiber tracts were significantly degenerated. The tracts that originated in the limbic and association nuclei exhibited the worst levels of disruption and the strongest association with cognition. The integrity of the fiber tracts alone was not sufficient to discriminate between the differeing levels of cognitive dysfunction in the PD samples however, it was only when the integrity of the thalmic nuclei was also considered that a high accuracy of identification of cognitive dysfunction was possible. We showed that PD-D especially, could be identified from PD-N with 74 - 86% accuracy. More importantly however, PD-MCI could be identified from PD-N with 71 - 77% accuracy. This is especially relevant in the current medical climate as several different recommendations for the clinical criteria for PD-MCI have been made (Aarsland, et al., 2010; Litvan, et al., 2011; Petersen, et al., 1999), and our group has shown some of these to be more lenient than others (Dalrymple-Alford, et al., 2011). The current thesis could go some way in aiding understanding of the progression from PD-MCI to PD-D and aid in identification of the neuropsychological criteria best applied to identify this. Although ~ 70 – 85% accuracy may not be as high as desired it should be noted that the predictive strength of mammograms for detecting breast cancer in the general population is also within this range, at 76% and this method is the current gold standard for breast cancer detection and monitoring (National Collaborating Centre for Cancer, 2009).

10.2 Limitations

As with any long term research, limitiations arose during the course of this thesis. We hoped that by applying a region of interest approach that some of the common pitfalls of neuroimaging such as spurious results or insensitivity to changes in small regions could be avoided. We feel this has mostly been achieved, and our structural T1 volume changes, especially are in accordance with other studies. The structural and diffusion imaging methods have been validated in an AD sample (de Jong, et al., 2008) and were carefully checked in order to ensure coherence with anatomical guidelines (Duvernoy, 1991; Nieuwenhuys, et al., 2008a). Our region of interest VBM approach has only been applied once before in PD (Summerfield, et al., 2005), using slightly different methodology and results here are not consistent as our sample reports no grey or white matter changes in PD. The lack of grey matter changes in the thalamus has previously been reported by our own group who applied a whole brain approach to a similar patient cohort (Melzer, et al., 2011b) so we feel that the region of interest approach has not resulted in grey matter change being 'missed' in this sample.

Diffusion tensor imaging is a relatively new technique, and despite multiple groups (Assaf & Pasternak, 2008; Chan, et al., 2007; Watts, 2008) reporting that increased mean diffusivity is the result of an increase in extracellular space due to the degeneration of neurons and supporting structures (Assaf & Pasternak, 2008), to the best of our knowledge this has not been verified by histology. Given that the cellular density and distribution of each thalamic nucleus is inherently variable (*See Chapter 4*) it could be argued that the variation in MD between nuclei is merely reflective of variations in cell density. The MDn, for example is made up of both widely dispersed cell bodies (anterior division) and smaller, packed cells (lateral division), and has a neuronal ratio (number of neurons/volume) 10 times that of the AP or CM/Pf nucleus (Henderson, et al., 2000b). In our sample, the MDn is one of the only regions to show a progressive increase in the PD group while the AP nucleus shows limited changes. We have included a control group for comparison; the MD increase in groups with greater cognitive dysfunction does represent a decline in cellular integrity and does not render results invalid. It cannot be overlooked however, that if a nucleus such as the MDn has a greater ratio of neurons to begin with then changes will be most evident here, regardless of underlying cause and may not indicate that the MDn is a target of degeneration, or is more affected than any other region.

It is also uncertain what FA values are actually representative of, with suggestions that myelin is not wholly reflected in this DTI measure. Reduced FA, instead may be the result of increases in extracellular space or, the increase in cellular water concentration (Assaf & Pasternak, 2008). Currently, it is difficult to explore this further as the DTI images are constrained by their large voxel size (in comparison to T1 images) and a $2.5mm^3$ voxel most likely contains tens of thousands of axons and glial cells. A single diffusion tensor could not represent all these components. Averaging cellular components presents with additional problems as areas of partial tissue volume, such as where grey matter and white matter are present in the same voxel will not be represented adequately by the DTI model (Jansons & Alexander, 2003; Papadakis, et al., 1999; Tuch, et al., 2002).

The main limitation centres on the fiber tracking approach. We used similar methodology to that that has been applied previously but as the majority of previous research has been conducted in a normal healthy sample it is difficult to establish what the effect of neurodegeneration would have had on the algorithm. Visual inspection of the images confirm what is known from previous animal studies and from what is suspected from fMRI and PET imaging studies, and is also in accordance with some of our own work (Melzer, et al., 2011a). We still do not know the direction of any of the fiber tracts however, as this is not yet a possibility using this DTI method (Leh, et al., 2007). Although the seed points originated subcortically and the tract was delinated outwards from here, the main white matter fibers could have been in any direction. Also, due to the abundance of white matter fibers within the thalamus, it is possible the algorithm picked up some tracts that in fact would have normally originated from another nucleus. This could potentially account for the frontal involvement seen here as the anterior thalamic radiation is a strong white matter pathway that could be dominating results. From the centromedian nucleus for example it is possible that instead of following white matter tracts that we would expect to diffuse in multiple directions it is possible the algorithm merley picked up the voxels in the strongest direction and followed the path from here, taping into the anterior thalamic radiation.

10.3 Recommendations

The thalamus is an intregral component of multiple neurological functions which mediate several facets of behavior. It is recommended that further research be carried out on a similar cohort to this one and followed longitudinally to validate the cross-sectional

findings of this thesis. Longitudinal investigation into these patients would allow for comparisions to be made between thalamic integrity in the early stages of the disorder when patients are still exhibiting cognitive function within the normal range and the assumed decline in integrity in those that progress to PD-MCI and finally to PD-D. Follow up analysis on this cohort may also provide a strong neurocorrelate of cognitive dysfunction which may aid in the earliest identification of cognitive decline in PD. Thalamic degeneration, especially in select areas could also be used to monitor any new pharmalogical or behavioural interventions that may aid in the treatment of cognitive impairment in PD in the future.

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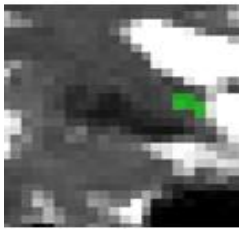
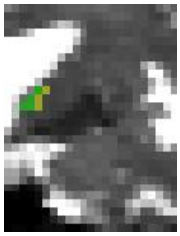
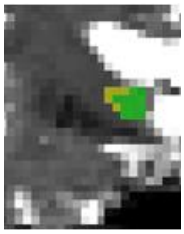
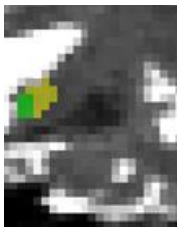
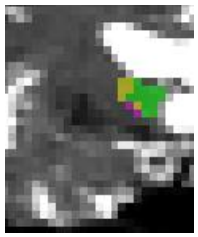
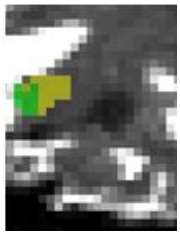
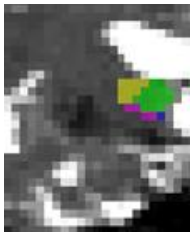
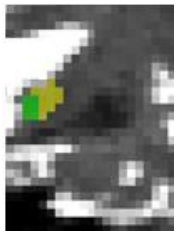
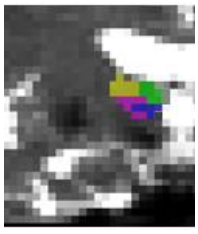
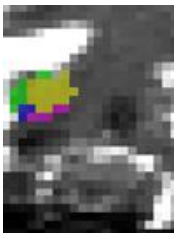
Chapter 12. Appendices

12.1 Appendix A: Nuclei Identification

Table 12-1: Coronal Slices

Right	Left	Nuclei
		AP (green) VA (yellow)
		AP (green) VA (yellow)
		AP (green) VA (yellow) VL (violet)
		AP (green) VA (yellow) VL (violet)
		AP (green) VA (yellow) VL (violet) CMPf (blue)

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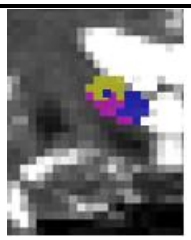
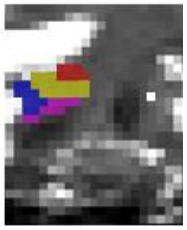
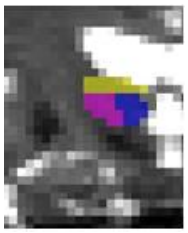
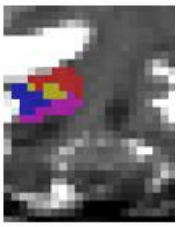
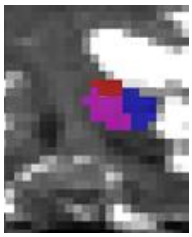
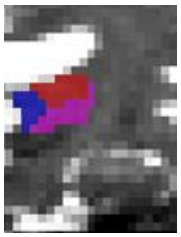
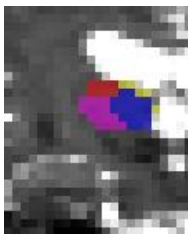
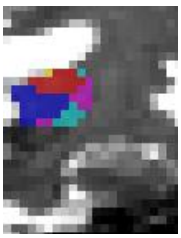
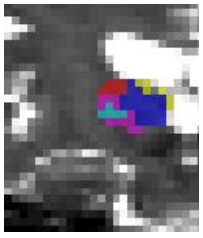
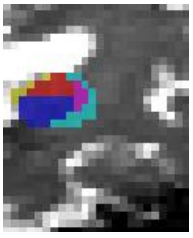
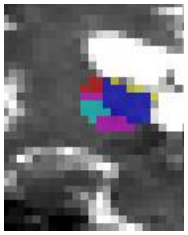
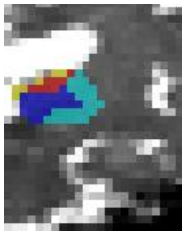
		VA (yellow) VL (violet) CMPf (blue) MD (red)
		VA (yellow) VL (violet) CMPf (blue) MD (red)
		VL (violet) CMPf (blue) MD (red)
		VL (violet) CMPf (blue) MD (red) LD (yellow) VPL (light blue)
		VL (violet) CMPf (blue) MD (red) LD (yellow) VPL (light blue)
		VL (violet) CMPf (blue) MD (red) LD (yellow) VPL (light blue)

Table 12-1 Continued

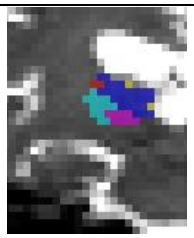
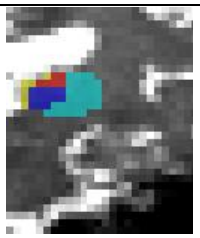
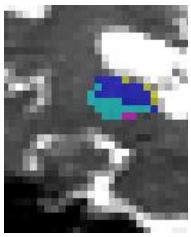
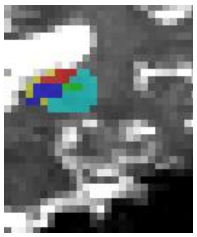
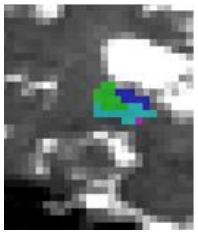
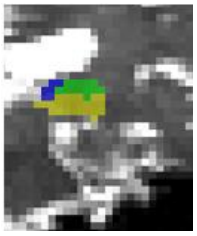
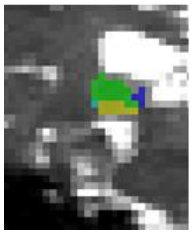
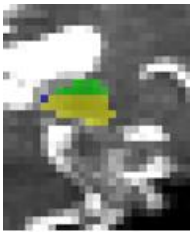
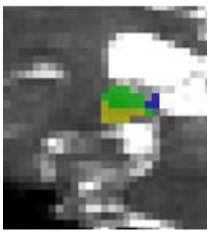
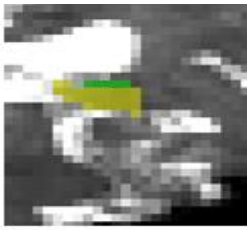
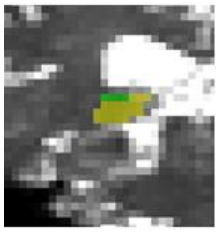
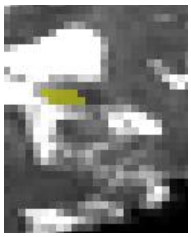
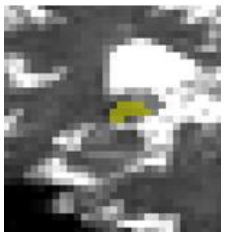
		VL (violet) CMPf (blue) MD (red) VPL (light blue) LD (yellow)
		CMPf (blue) MD (red) VPL (light blue) LD (yellow) LP (green)
		CMPf (blue) VPL (light blue) LP (green) Pulvinar (yellow)
		CMPf (blue) LP (green) Pulvinar (yellow)
		CMPf (blue) LP (green) Pulvinar (yellow)
		LP (Green) Pulvinar (yellow)
		Pulvinar (yellow)

Table 12-2: Saggital slices

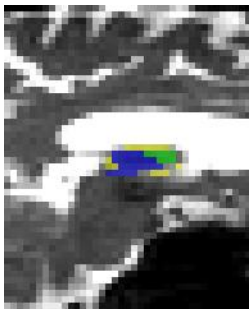
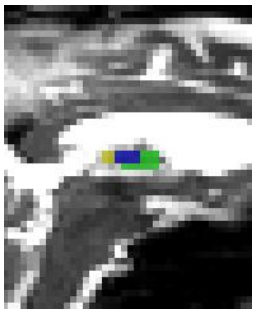
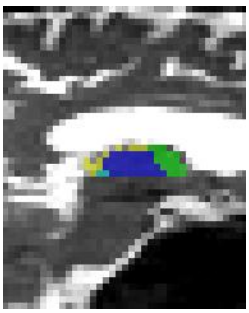
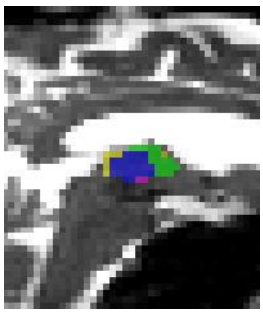
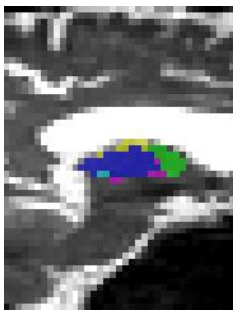
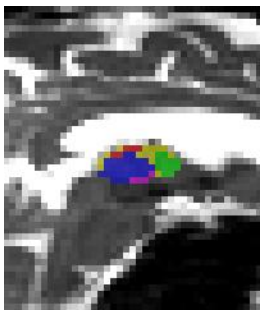
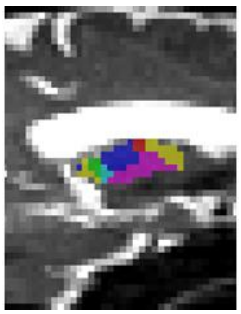
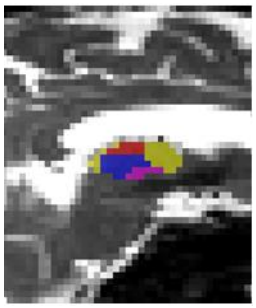
Right	Left	Nuclei
		LD (yellow) CMPf (blue) AP (green)
		LD (yellow) CMPf (blue) AP (green) VPL (light blue) VL (violet)
		LD (yellow) CMPf (blue) AP (green) VPL (light blue) VL (violet) MD (red)
		CMPf (blue) VPL (light blue) VL (violet) MD (red) VA (yellow) LP (green) Pulvinar (yellow)

Table 12-2 Continued

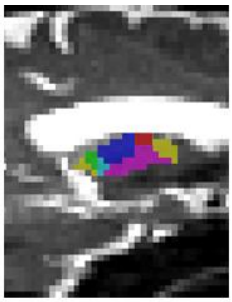
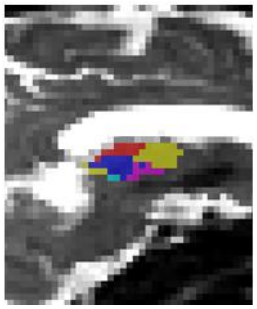
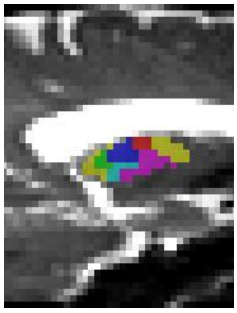
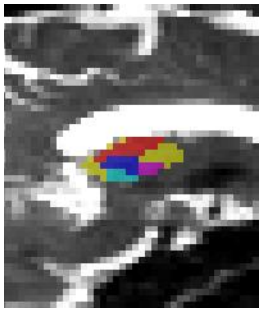
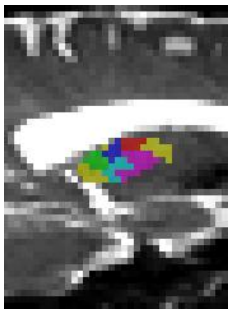
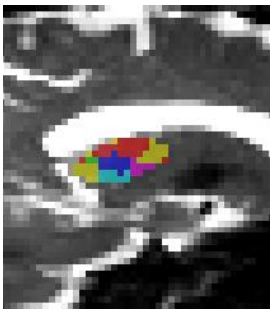
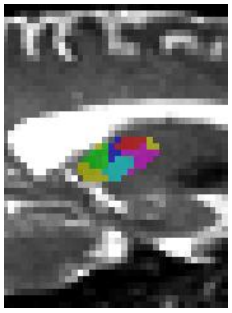
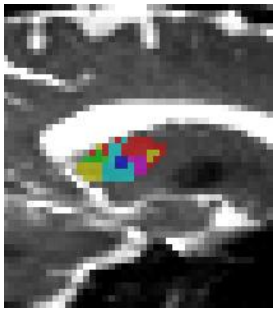
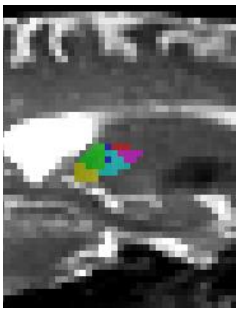
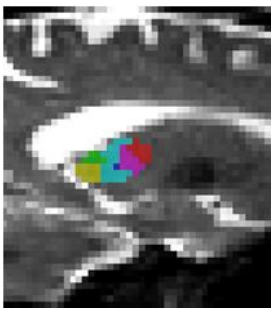
		CMPf (blue) VPL (light blue) VL (violet) MD (red) VA (yellow) LP (green) Pulvinar (yellow)
		CMPf (blue) VPL (light blue) VL (violet) MD (red) VA (yellow) LP (green) Pulvinar (yellow)
		CMPf (blue) VPL (light blue) VL (violet) MD (red) VA (yellow) LP (green) Pulvinar (yellow)
		CMPf (blue) VPL (light blue) VL (violet) MD (red) VA (yellow) LP (green) Pulvinar (yellow)
		CMPf (blue) VPL (light blue) VL (violet) MD (red) LP (green) Pulvinar (yellow)

Table 12-2 Continued

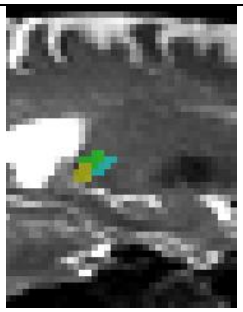
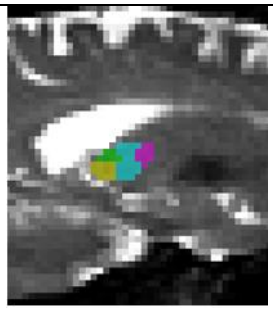
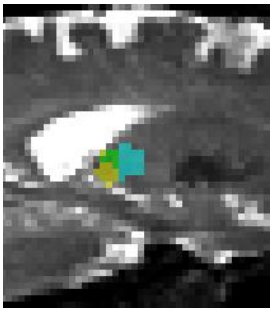
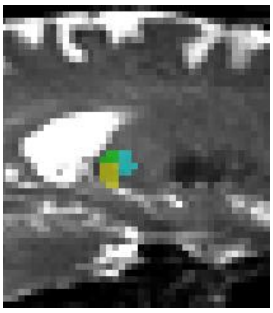
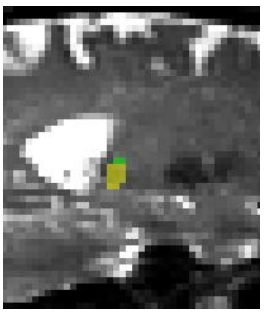
		VPL (light blue) VL (violet) LP (green) Pulvinar (yellow)
		VPL (light blue) LP (green) Pulvinar (yellow)
		VPL (light blue) LP (green) Pulvinar (yellow)
		LP (green) Pulvinar (yellow)

Table 12-3: Horizontal slices

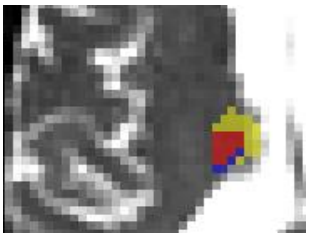
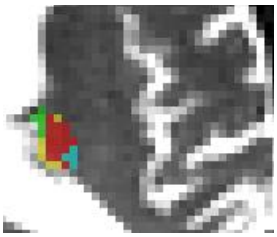
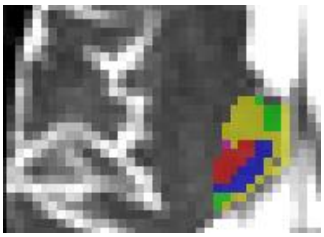
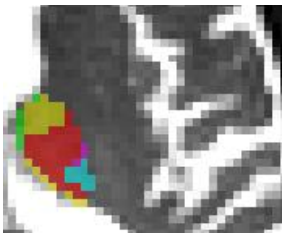
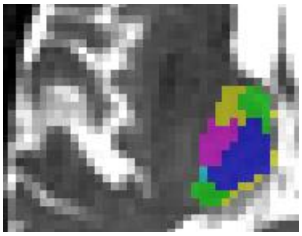
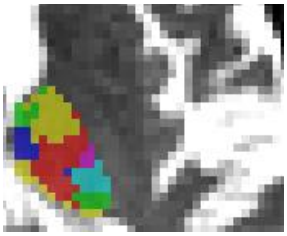
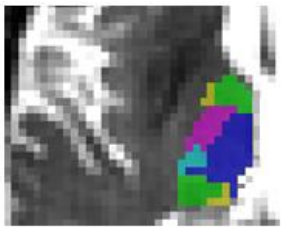
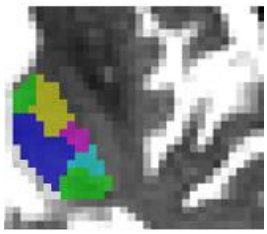
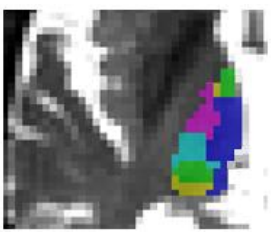
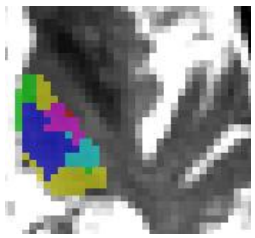
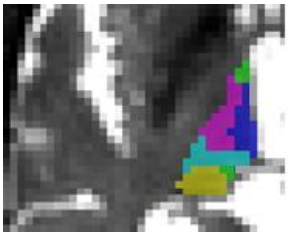
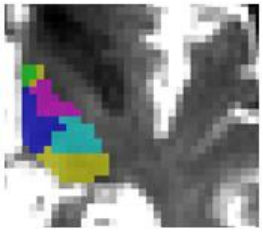
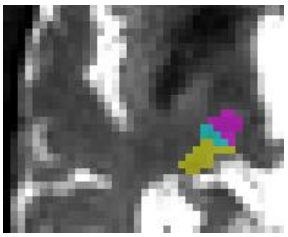
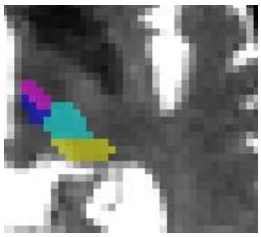
Right	Left	Nuclei
		AP (green) LD (yellow – medial) MD (red) VA (yellow – lateral) VPL (light blue)
		AP (green) LD (yellow – medial) MD (red) VA (yellow – lateral) VL (violet) VPL (light blue) CMPf (blue)
		AP (green - anterior) LD (yellow – medial) MD (red) VA (yellow – lateral) VL (violet) VPL (light blue) CMPf (blue) LP (green – posterior) Pu (yellow – posterior)
		AP (green - anterior) VA (yellow – lateral) VL (violet) VPL (light blue) CMPf (blue) LP (green – posterior) Pu (yellow – posterior)
		AP (green - anterior) VA (yellow – lateral) VL (violet) VPL (light blue) CMPf (blue) LP (green – posterior) Pu (yellow – posterior)

Table 12-3 Continued

		AP (green - anterior) VA (yellow – lateral) VL (violet) VPL (light blue) CMPf (blue) Pu (yellow – posterior)
		VL (violet) VPL (light blue) CMPf (blue) Pu (yellow – posterior)

12.2 Appendix B: Logistic regression results

Table 12-4: Combined effect of tracts and nuclei using FA and MD Logistic regression results, predicting PD

	Association			Limbic		Sensory	Non-Specific	Motor		Whole Thalamus		
	MDn	LP	Pu	AP	LD	VP	CM/Pf	VA	VL	Left	Right	Average
C vs PD-N												
ROI												
FA	-0.14	-0.34	-0.27	-0.25	-0.33	-0.28	-0.66	-0.09a	-0.17	-0.47	-0.32	-0.40
MD	-0.74	-0.60	-0.71	-0.64	-0.26	-0.72	-0.68	-0.70	-0.76	-0.81	-0.78	-0.81
Tract												
FA	-0.66	-0.45	-0.54	-0.68	-0.65	-0.40	-0.28	-0.66a	-0.43	-0.40	-0.49	-0.41
MD	-0.25	-0.37	-0.28	-0.38	-0.62	-0.16	-0.45	-0.12	-0.20	0.15	0.13	-0.16
Model												
chi^2 (df=4)	5.11	0.90	14.54	6.37	4.50	4.39	2.66	11.87	4.47	2.91	2.17	2.49
p	0.28	0.92	0.01	0.17	0.34	0.36	0.62	0.02	0.35	0.57	0.70	0.65
% correct	69.33	68.00	76.00	66.67	64.00	68.00	69.33	73.33	68.00	69.14	69.14	69.14
C vs PD-MCI												
ROI												
FA	-0.13	-0.26	-0.30	-0.19	-0.03	-0.36	-0.38	-0.30	-0.12	-0.66	-0.59	-0.62
MD	-0.39a	-0.45	-0.63a	-0.33	-0.02	-0.63	-0.63	-0.76a	-0.65	-0.82	-0.86	-0.85
Tract												
FA	-0.71	-0.53	-0.59	-0.73	-0.80	-0.34	-0.46	-0.70	-0.51	-0.45	-0.47	-0.47
MD	-0.67	-0.52	-0.30	-0.68	-0.67	-0.27	-0.42	-0.21	-0.35	0.37	0.23	0.33
Model												
chi^2 (df=4)	8.28	3.09	9.22	2.15	9.06	3.33	2.28	8.36	3.58	9.02	7.89	8.57
p	0.08	0.54	0.06	0.71	0.06	0.50	0.69	0.08	0.47	0.61	0.10	0.07
% correct	66.67	59.52	74.43	59.52	50.00	66.67	59.52	71.42	59.52	68.18	63.63	65.91
C vs PD-D												
ROI												
FA	-0.25	-0.12	-0.35	-0.58	-0.20	-0.04	-0.41	-0.37	-0.11	-0.59a	-0.47	-0.53
MD	-0.78	-0.66	-0.69	-0.24a	-0.28	-0.76a	-0.85	-0.64	-0.83a	-0.83	-0.86a	-0.84a
Tract												
FA	-0.66	-0.63	-0.71	-0.58	-0.62	-0.43	-0.70	-0.56	-0.08	-0.30	-0.40	-0.33
MD	-0.56	-0.51	-0.26	-0.77a	-0.59	-0.51	-0.82	-0.30a	-0.45	-0.05a	-0.26a	-0.16b
Model												
chi^2 (df=4)	19.59	6.72	16.35	15.17	9.98	18.03	24.27	15.64	19.25	23.83	25.60	25.10
p	>0.01	0.15	>0.01	>0.01	0.04	>0.01	>0.001	>0.01	>0.001	>0.001	>0.001	>0.001
% correct	84.62	71.79	87.18	76.92	66.67	82.05	84.62	76.92	74.36	82.05	82.05	82.05

ROI = region of interest (either nucleus or whole thalamus). Superscript numbers denote significance at the following levels: a: $p < 0.05$; b: $p < 0.01$; c: $p < 0.001$.

Table 12-5: Combined effect of tracts and nuclei using FA and MD Logistic regression results, predicting cognitive impairment

	Association			Limbic		Sensory	Non-Specific	Motor		Whole Thal		
	MDn	LP	Pu	AP	LD	VP	CM/Pf	VA	VL	Left	Right	Average
PD-N vs PD-MCI												
ROI												
FA	-0.30	-0.37	-0.36	-0.12	-0.37	-0.37	-0.55	0.06	0.07	-0.40	-0.28	-0.34
MD	-0.73	-0.57	-0.52	-0.43	-0.18	-0.59	-0.55	-0.50	-0.48	-0.71	-0.78	-0.76
Tract												
FA	-0.55	-0.52	-0.64	-0.67	-0.72	-0.41	-0.48	-0.76	-0.65	-0.42	-0.53	-0.45
MD	-0.09	-0.33	-0.36	-0.51	-0.68	-0.16	-0.37	-0.44	-0.43	0.16	0.13	0.18
Model												
chi^2 (df=4)	7.85	2.97	7.59	4.02	9.72	4.35	1.97	10.82	6.61	9.36	7.09	8.53
p	0.97	0.56	0.11	0.40	0.05	0.36	0.74	0.03	0.16	0.05	0.13	0.07
% correct	72.46	73.91	76.81	71.01	72.46	73.91	73.91	76.81	75.36	77.33	77.33	77.33
PD-N vs PD-D												
ROI												
FA	-0.30	-0.37	-0.32	-0.11	-0.41	-0.16	-0.37	0.05	0.20a	-0.37	-0.20	-0.31
MD	-0.62a	-0.68b	-0.48	-0.14	-0.22	-0.58	-0.49	-0.52	-0.39	-0.73a	-0.80a	-0.77b
Tract												
FA	-0.54	-0.60	-0.66	-0.74	-0.56	-0.46	-0.74	-0.72	-0.64	-0.41	-0.50	-0.42
MD	-0.35	-0.36	-0.53	-0.71	-0.74	-0.43	-0.74	-0.10c	-0.75b	-0.14a	-0.01a	-0.06a
Model												
chi^2 (df=4)	20.53	20.53	12.98	17.41	18.10	14.22	20.65	28.78	18.44	23.98	24.31	25.09
p	>0.01	>0.001	0.01	>0.01	>0.01	>0.01	>0.01	>0.001	>0.01	>0.001	>0.001	>0.001
% correct	80.30	74.24	80.30	78.79	75.76	80.30	80.30	86.36	83.33	84.93	82.19	84.93
PD-MCI vs PD-D												
ROI												
FA	-0.20	-0.35	-0.45	-0.19	-0.16	-0.38	-0.16	-0.17	0.14	-0.67	-0.72	-0.70
MD	-0.44	-0.56	-0.40	-0.07	-0.24	-0.41	-0.75a	-0.49a	-0.48	-0.71	-0.84	-0.79
Tract												
FA	-0.62	-0.73	-0.71	-0.83	-0.82	-0.37	-0.80	-0.68	-0.55	-0.28	-0.44	-0.35
MD	-0.51	-0.48	-0.46	-0.84	-0.79	-0.41	-0.42	-0.07	-0.29	0.12	-0.03	0.11
Model												
chi^2 (df=4)	8.44	5.13	3.08	12.31	7.22	5.86	17.26	12.15	5.61	4.66	7.60	5.73
p	0.08	0.27	0.54	0.02	0.12	0.21	0.002	0.02	0.23	0.32	0.11	0.22
% correct	75.76	66.67	66.67	72.72	66.67	66.67	69.70	66.67	66.67	66.67	63.89	66.67

ROI = region of interest (either nucleus or whole thalamus). Superscript numbers denote significance at the following levels: a: $p < 0.05$; b: $p < 0.01$; c: $p < 0.001$.